

ACTA MEDICA SCANDINAVICA

*

REDACTORES

<i>ERIK ASK-UPMARK</i> UPSALA	<i>GÖSTA BECKER</i> HELSINGFORS	<i>G. BERGMARK</i> UPSALA	<i>H. I. BING</i> KÖBENHAVN
<i>BERTEL VON BONSDORFF</i> HELSINGFORS	<i>R. EHRSTRÖM</i> HELSINGFORS	<i>K. FABER</i> KÖBENHAVN	<i>OLAV HANSEN</i> OSLO
<i>ÖSTEN HOLSTI</i> HELSINGFORS	<i>C. HOLTEN</i> AARHUS	<i>LAURI KALAJA</i> ÅBO	<i>WILLIAM KERPPOLA</i> HELSINGFORS
<i>ANDERS KRISTENSON</i> STOCKHOLM	<i>CARL MÜLLER</i> OSLO	<i>EGGERT MÖLLER</i> KÖBENHAVN	<i>F. SALTZMAN</i> HELSINGFORS
<i>H. A. SALVESEN</i> OSLO	<i>JÓN HJ. SIGURÐSSON</i> REYKJAVÍK	<i>NANNA SVARTZ</i> STOCKHOLM	
<i>HANS JACOB USTVEDT</i> OSLO	<i>JAN WALDENSTRÖM</i> UPSALA	<i>ERIK WARBURG</i> KÖBENHAVN	
<i>J. G. G. BORST</i> AMSTERDAM	<i>F. S. P. VAN BUCHEM</i> GRONINGEN	<i>P. FORMIJNE</i> AMSTERDAM	
<i>C. D. DE LANGEN</i> UTRECHT		<i>J. MULDER</i> LEYDEN	

EDITOR

I. HOLMGREN
STOCKHOLM

COLLABORANT:

- IN DANIA: S. Bang, H. C. Gram, Poul Iversen, Aage Th. Jacobsen, Ejnar Jarlov, E. Meulengracht, Otto Moltke, A. Norgaard, Aa. Nyfeldt, K. Schroeder, K. Secher.
- IN FENNIA: Erik Adlercrentz, Pekka Brummer, Mons-Christian Ehrström, Martti Hirvonen, Martin Savolin, Pauli Soisalo, Guido Tötterman, I. Vartiainen, J. Wahlberg, E. A. v. Willebrand.
- IN HOLLANDIA:
- IN NORVEGIA: Olaf Bang, Gunnar Bøe, R. Hatlehol, Fr. Harbitz, H. F. Host, Anton Jervell, G. H. Monrad-Krohn, K. Motzfeldt, Olaf Rønneke
- IN SUECIA: Hilding Berglund, Stig Björkman, Leonard Brahme, Gustaf Brun, Arthur Engel, Birger Enocksson, Claes Grill, A. Gullbring, Sixten Hesser, G. Kahlmeter, Kj. O. af Klercker, Oscar Lindeboom, Malte Ljungdahl, Haqvin Malmros, Gustav Nylin, Martin Odén, Ernst Sahlgren, Ernst B. Salén, Elsa Segerdahl, Birger Strandell, J. Tillgren, Erik Wassén, A. Westergren, H. Öhnell.

ACTA MEDICA SCANDINAVICA'S FÖRLAG, STOCKHOLM

INDEX.

Vol. CXXXI.

	Pag.
OLE JACOB BROCH (Tonsberg, Norway): Premortal changes in the concentration of the serum electrolytes	1
POUL EFFERSØE (Frederiksborg, Denmark): Nephrectomy in hypertension and unilateral renal disorder	10
C.-A. ADAMSON and GÖSTA HAGERMAN (Stockholm): On the treatment of actinomycosis with sulpha drugs and penicillin	23
HANS JACOB USTVEDT (Oslo): Erythema exsudativum multiforme. I	32
A. J. M. LOHMAN (Weert, Holland): Mediastinitis anterior chronica .	51
TAGE GRINSTED (Odense, Denmark): Titration of serum with mercuric chloride especially in liver affections	66
LADISLAUS MOSONYI und ELISABETH OBLATT (Budapest): Störungen im Vitaminhaushalt bei längerer Penicillinbehandlung	82
TORBEN K. WITH (Copenhagen): Note on the pathogenesis of jaundice	93
HOLGER BEGTRUP (Copenhagen): Correction to: Pancreatic disease combined with Vitamin-K-refractory hypoprothrombinemia . . .	95
PETTER KARLBERG (Stockholm): The accuracy of some clinical methods (Autenrieth, Stufen and Sicca) of determining hemoglobin values — a comparison	99
B. SWEDBERG and G. WIDSTRÖM (Stockholm): Treatment of experimental tuberculosis in mice and guinea-pigs with para-aminosalicylic acid (PAS) and streptomycin	116
KNUD BRØCHNER-MORTENSEN, HANS CHR. ENGBÆK and KAI SCHMITH (Copenhagen): Specific serum treatment of Pfeiffer meningitis . .	129
HANS CHR. ENGBÆK (Copenhagen): Pfeiffer meningitis, treated with streptomycin, sulphonamide and type-specific rabbit immune serum	146
G. A. GUSSENHOVEN (Voorburg, The Netherlands): Sulphasuccidine a cause of polyneuritis	159
GEORGES DE BUSSCHER (Bruges, Belgique): L'endartérite oblitérante et l'endophlébite en cas d'ulcère de l'estomac	164
INGER-LOUISE MARNER (Copenhagen): The thymol reaction as a liver test	180
IMRE MAGYAR and PAUL RESOFSZKI (Budapest): The effect of thiamine (vitamin B ₁) on the utilisation of carbohydrates by the tissues . .	193
VAGN MORTENSEN and ERIK WARBURG (Copenhagen): Chronic constrictive pericarditis	203
HANS JACOB USTVEDT (Oslo): Erythema exudativum multiforme. II.	226
NILS ALWALL and LEMBIT NORVIIT with the collaboration of A. M. STEINS (Lund, Sweden): On the artificial kidney. III	237
BENGT SKANSE (Boston, U. S. A.): Radioactive iodine: its use in studying the urinary excretion of iodine by humans in various states of thyroid function	251

F. MAINZER (Alexandria, Egypt): The pellegra-electrocardiogram and its significance	269
RICH. AMLIE and PER OEDING (Oslo): The clinical value of the anti-streptolysin reaction	288
LISA BOSTRÖM (Stockholm): Are non-nucleated erythrocytes formed by budding off of cytoplasm from normoblasts?	303
OLE STORSTEIN (Bergen, Norway): The value, as a clinical test, of the titration of aneurin in the urine	325
HARALD A. SALVESEN (Oslo): The effect of blood transfusions on the kidney function of chronic nephritis with anemia	337
St. J. LEITNER unter Mitarbeit von W. THALMANN (Heiligenschwendi, Schweiz): Untersuchungen über die Kantharidenblasenreaktion bei Tuberkulose	342
G. A. LINDEBOOM (Amsterdam): Hyperglobulinemia and pregnancy	368
ERIC JONSSON, LISA BOSTRÖM and BIRGER BRINGEL (Stockholm): Pelger-Huët's anomaly of the nuclei of the leucocytes	380
SVERRE AARSETH (Haugesund, Norway): Melorheostosis	394
HUGO CHIODI (Buenos Aires): Variability of the lung volume measurements in patients with pulmonary tuberculosis	403
PER HEDLUND, A. RUNE FRISK and HÄRJE BUCHT (Stockholm): The appearance of acute phase protein after induced fever in man	417
RUBEN GORDIN (Helsingfors): Alternating nodal and sinus rhythm in a case of situs viscerum inversus	422
J. MULDER and W. R. O. GOSLINGS, assisted by S. W. ENSERINK (Leyden, The Netherlands): Simplified micro-Hirst technique	431
FADEUSZ TEMPKA and MIECZYSLAW KUBICZEK (Cracow, Poland): Normal and pathological lymphadenogram in the light of own research	431
HENRIK F. LANGE and HERBERT PALMER (Oslo): Studies of erythrocyte counting. I.	451
OLLE HOGEMAN (Upsala, Sweden): Family epidemic of primary atypical pneumonia	466
HAROLD S. GINSBERG (New York): Serological studies on a family epidemic of primary atypical pneumonia	475
ORAZIO CARERE-COMES and ALBERTO TESI (Florence, Italy): Tuberculin test and serum antibodies in the experimental tuberculosis and their behaviour in order to the anatomical evolution of the disease	481
C. H. BEST (Toronto, Canada): The lipotropic factors	503
HALVOR VERMUND (Tonsberg, Norway): The relation of hypophysis to carbohydrate and basal metabolism	515
P.H.D. WAAGSTEIN (Maribo, Denmark): A case of perniciosiform anemia in a child nineteen months old	547
HENRIK F. LANGE and HERBERT PALMER (Oslo): Studies of erythrocyte counting. II.	555
EBBE NYMAN (Stockholm): Comparative studies of the effect of some vasodilators in angina pectoris	565
GUNNAR EDSTRÖM (Lund, Sweden): Chrysotherapy and its toxic reactions in rheumatoid arthritis	571
INGA LINDGREN and A. RUNE FRISK (Stockholm): The effect of tetraethylammonium ion in arteriosclerotic heart disease	581
SVEN MUNCH-PETERSEN (Aarhus, Denmark): On serum copper in angina simplex and in infectious mononucleosis	588

GÖSTA BJÖRKENHEIM (Helsingfors): On auricular fibrillation and block, in connection with a recent case	597
VINCENT MUTOLO (Palerme, Italie): Sur la différenciation des sérums ictériques néoplasiques et non néoplasiques	602

Book review:

WILH. WERNSTEDT (Stockholm): M. Tramer: Lehrbuch der allgemeinen Kinderpsychiatrie	96
---	----

Index of the whole series of supplementary volumes, published 1921 —1948	605
---	-----

Supplementum CCXII (212), ILMARI PARONEN (Helsingfors): Reiter's disease.

Supplementum CCXIII (213), EINAR MEULENGRACHT, in honorem.

Supplementum CCXIV (214), AXEL STRÖM (Oslo): Examination into the diet of norwegian families during the war-years 1942—45.

From Vestfold County Hospital Medical Department,
Tønsberg (Norway).
(Chief Physician: Anton Jervell, M. D.)

Premortal Changes in the Concentration of the Serum Electrolytes.

By

OLE JACOB BROCH.

Oslo.

(Submitted for publication October 8, 1947.)

In a previous investigation respecting the regulation of the serum electrolytes (Broch 1945) there was found in some patients a striking premortal rise in chloride and total bases in the serum.

In one particular case the total bases rose by 25 m.eq. in the course of one week before death. No explanation of this phenomenon was given. The total bases may vary considerably in certain pathological conditions and such variations are due, practically speaking, to the Na ion alone. As a rule, reduced values are noted. The greatest changes are found in case of renal insufficiency, as has been shown by Salvesen in an extensive investigation in 1928. In 7 out of 87 cases of nephritis Peters, Wakeman, Eisenman and Lee found values above the normal, in one case nearly 200 m.eq. The importance of the kidneys for the distribution of electrolytes in the organism has been studied experimentally by Iversen and Hansborg, among others. After ligation of the ureter Atchley and Benedict found acidosis with increase in sulphates and phosphates, but no characteristic changes of the total bases.

Allot has described four cases, without renal changes, with very high Na and Cl values in serum (total bases up to 179 m.eq./

L). Three of these patients had lesions in the central nervous system and all died relatively soon after the investigations. He also found increased content of urea in the blood. It is reasonable to suppose that these were the same premortal changes as are spoken of above in the introduction. Allot mentions that clinical signs of dehydration were noted, but thinks that the explanation perhaps is that the cerebral lesion leads to increased secretion of pituitrin and thereby to increased re-absorption in the tubules.

An extensive study on the agonal acidosis was newly published by Fabricius Hansen. He found the total bases as usual abnormal. Mostly the total bases were high and in several cases above 170 m.eq./L. He also found high values of N. P. N. and the undetermined acids in serum.

The increase in content of salt in the blood can be supposed to arise through dehydration, with loss especially of water. But in the states of dehydration usually met with in clinical practice such electrolytic changes do not occur.

In another investigation (Broch 1946) I have studied the behaviour of the electrolytes in 19 patients without affections of the kidneys in whom there was seen desiccation due to various causes. The general condition was in most of them comparatively little affected. They all received adequate fluid-therapy. Only three of the patients showed a moderate rise in total bases (highest value: 162.8 m.eq./L). In the great majority the total bases were reduced and fell as a rule still lower after ingestion of liquid. Subnormal values could be noted for up to three weeks, in spite of sufficient supply of salt and liquid. The serum chloride content depended on the disturbances of the acid-base equilibrium.

In the present publication the premortal electrolytic changes have been taken up for renewed examination, especially with a view to the importance of dehydration.

Particulars respecting the material investigated will be found in the table. It comprises 14 patients in all, whereof 12 died in the hospital. Eight of the patients were suffering from vascular cerebral lesions, which, except in two cases, led to death in the course of comparatively few days. The first four were cases of fatally resulting apoplexy, in all of which there was seen a rise in the total bases, sometimes considerable, being most pronounced in Case 1. Two days after the hemorrhage the total bases here showed a distinct increase and on the day before death the value noted was 184.1 m.eq./L. There was moderate acidosis. The undetermined

acids (total bases — $(\text{Cl} + \text{HCO}_3 + \text{base equivalent of proteins})$)¹ were found at the first examination, 22/10 to amount to 23.2 m.eq., while on the day before death they rose to 38.9 m.eq. The difference must be due to pathological organic acids. The hematocrit value increased from 35 to 49 and there was noted a corresponding rise in hemoglobin and erythrocytes. At the same time there was also seen a rise in N. P. N. Corresponding conditions were found in the other cases. In Case 3 the total bases rose by 25.5 m.eq., while Cl and HCO_3 increased by only 12.1 m.eq. There was considerable dehydration and the N. P. N. increased three-fold.

These changes were noted in all cases of fatal apoplexy. The changes may come on rapidly, after no more than twenty-four hours (Case 4). A case of traumatic intracranial hemorrhage (No. 8), with death within the first twenty-four hours, showed, however, normal electrolytic conditions. In the patients who survived the injury the phenomenon was not noted at all, or at most merely a suggestion thereof. One of them (No. 6) was comatose for less than one day. The other (No. 7) had paresis of the gullet, but no impairment of consciousness. She consumed very little during the first time and the total bases lay slightly above the normal. By degrees there came a distinct reduction, according as she was supplied with liquids. The examinations seemed to show a slight degree of hemoconcentration. The material does not comprise many different causes of death, but the same electrolytic changes with considerable acidosis were also found in marked degree in a case of acute psychosis (No. 9). The patient was very restless, took no nourishment and was considerably dehydrated. These changes represent at any rate no decided premortal phenomenon. In three patients with protracted cachexia and extensive edema (Nos. 12, 13 and 14) the total bases were rather found to be somewhat reduced. None of these patients showed signs of dehydration and the N. P. N. values were practically normal. No. 10 had cardiac infarction and died rather suddenly in circulatory collapse, with normal total bases and N. P. N. and without signs of dehydration immediately before death. The last patient, No. 11, who had bronchopneumonia, was examined three days before death. It must be deemed probable that the same changes

¹ The base equivalent of the proteins was calculated after Van Slyke and co-workers. In pathological cases such calculation will be extremely uncertain, as the base-binding power of the proteins may vary considerably, independent of quantitative variations in the proteins (Broch 1945).

would have been found in this case, if the analyses had been repeated at a later point of time.

Discussion.

It can hardly be doubted that the necessary requirement for the production of these premortal electrolytic changes is a considerable degree of dehydration, leading at the same time to azotemia and pathological acids in the blood. The supply of liquids was minimal and the diuresis was impossible to measure. It is understandable that these changes will be most pronounced in case of apoplexy, where the patients are lying unconscious and receive no nourishment and where parenteral administration of liquids is usually omitted in view of the hopeless prognosis.

The cause must be supposed to be a selective loss of water, together with some retention of salt. Judging from the hemoconcentration the loss of water is percentually far greater than the increase in the total bases. The patients have been highly febrile and the insensible loss of water has certainly been considerable. Some have perspired very profusely and the sweat contained relatively little salt. One patient (No. 5) was given one litre of a 2.6 per cent sodium bicarbonate solution intravenously in two days. He got a cerebral thrombosis, became soporous and on the next day comatose. He died after four days. The conditions for an increase of the total bases, such as was seen in the first patients, should here be supposed to exist. In spite of intravenous injection of about 620 m.eq. of Na in the form of bicarbonate, which about corresponds to the whole content of Na in the blood, there came in this case a reduction of the serum Na. Neither was there any hemoconcentration, which probably explains why no premortal rise in the total bases occurred in this case.

The great degree of dehydration and the impaired vitality must be supposed probably through reduced filtration, to put the kidney partly out of action. The retention of urea and pathological acids may also be said to point in that direction. Perhaps there may be an increased reabsorption of Na through the tubules.

No increase of electrolytes was noted in patients with edema (Nos. 12, 13, 14). It is reasonable to suppose that the abundant supply of extracellular fluid serves as a depot which stands at disposal and prevents dehydration and reduction of the blood volume.

That these changes are in general not seen in case of an ordinary reversible clinical dehydration must be supposed to be due to the renal function not being sufficiently impaired.

Case Histories.

1. Jr. No. 4919. Woman aged 73. Hypertension without cardiac insufficiency. $^{20}/_{10}$ 1946: Apoplexy with left-sided hemiplegia. Admitted same day. B. P. about 255/140, frequent fibrillation arrhythmia. Partly comatose. Liquid per os: $^{21}/_{10}$: 450 cc, $^{22}/_{10}$: 300 cc, $^{23}/_{10}$: 250 cc, $^{24}/_{10}$: 200 cc, $^{25}/_{10}$: 50 cc. Last two days high fever. Died $^{27}/_{10}$.

2. Jr. No. 5102. Woman aged 63. For many years hypertension with slight cardiac insufficiency. $^{30}/_{10}$ 1946, 4 p. m. apoplexy with left-sided hemiplegia. Admitted same day. Since then mostly comatose. Very scanty ingestion of liquids. $^{3}/_{11}$: 1000 cc saline intravenously, as well as 1000 cc subcutaneously. Died a few hours later.

3. Jr. No. 5471. Woman aged 74. For many years »rheumatism» in knees and wrists. »Weak heart». $^{21}/_{11}$ 1946: Apoplexy with sudden unconsciousness. Admitted same day. Was soporous, aphasia. B. P.: 160/95, fibrillation arrhythmia. $^{22}/_{11}$: Just barely reacts when spoken to. Urine passed in bed all the time. Ingestion of liquids about 600—900 cc daily. $^{2}/_{12}$: 1500 cc saline intravenously. Died same day in high fever.

4. Jr. No. 5857. Man aged 86. Fibrillation arrhythmia for many years. In last weeks dyspnea on exertion. Admitted $^{17}/_{12}$ 1946. Fibrillation arrhythmia with frustraneous contractions. Moderate edema. Congestion of lungs. Was given digitalis and mercury diuretics. $^{18}/_{12}$ 1946: Hgb. 88 %. Erythrocytes: 1.48 mill./cc. $^{3}/_{1}$ 1947: Apoplexy with right-sided hemiplegia. Very scanty ingestion of liquids. Rise of temp. to 41° C. Died $^{5}/_{1}$ 1947.

5. Jr. No. 12766. Man aged 67. Many years of insulin treatment for diabetes mellitus. Admitted $^{21}/_{8}$ 1947 for gangrene of right foot. $^{11}/_{8}$: Amputation of leg from middle of thigh. Since then phlegmon with constant secretion from amputation stump. Subfebrile and gradually cachectic. Fibrillation arrhythmia with moderate cardiac disturbance. $^{1}/_{8}$: Increasingly soporous, with aphasia and slow cerebration. Assumed thrombosis cerebri. Temperature rose to 41° C. and he became completely comatose. In the last couple of days almost no ingestion of liquids per os. $^{2}/_{8}$ and $^{3}/_{8}$: 1000 cc 2.6 % NaHCO_3 intravenously each day. Died $^{5}/_{8}$ 1947.

6. Jr. No. 5269. Man aged 68. For many years hypertension without cardiac insufficiency. $^{3}/_{4}$ 1945: A temporary cerebral attack, regarded as thrombosis. Slight psychic impairment. $^{10}/_{11}$ 1946: Suddenly lost consciousness. Admitted same day. B. P. 200/110. Cardiac disturbance without signs of insufficiency. $^{10}/_{11}$: General cramps, with unconsciousness of some hours' duration. On some days slightly soporous. Gradually regained full clarity. Discharged $^{22}/_{11}$ 1946.

¹ From Rikshospital, Med. Dept. B. Chief: Prof. H. A. Salvesen, M. D.

Case	Date	Total bases	Cl.	HCO ₃	Total protein	Albu- min	Glo- bulin	N.P.N. mg/100 ml	Hgb.
1	²² / ₁₀	165.6	107	21	7.38	4.30	1.40	63.4	90
	²² / ₁₀	165.9	100	21	7.38			66.4	
	²³ / ₁₀	177.7	99	21.5	7.18			100.2	110
	²⁶ / ₁₀	184.1	107.5	18.9	8.06	4.26	3.80	147.1	133
2	³¹ / ₁₀	165.2	79.5	20.6	9.58	5.69	3.89	74.2	124
	² / ₁₁	165.4	96.5	22.3	8.90	5.24	3.66	78.1	133
	11.30 a. m.								
	³ / ₁₁	171.1	103	21.5					130
	7.15 p. m.								
	³ / ₁₁	169.3			7.51	4.21	3.31	134.1	128
3	²² / ₁₁	155	95	24	7.40	4.26	3.14	41.7	88
	² / ₁₂	180.5	105	26.1	8.60	4.07	4.53	134.5	119
4	⁴ / ₁	182.6	86	24.9	9.12	4.89	4.23	54.7	120
5	² / ₈			15.6					78
	³ / ₈	155.5	86						77
	⁴ / ₈	149	87	31.8				84 ¹	80
6	¹¹ / ₁₁	157	93	26.6	7.65				104
	¹² / ₁₁	152.1	83.5	26.6	6.93				106
7	²² / ₁₁	160.7	89	27.4	7.29	3.74	3.55	43.8	70
	²³ / ₁₁	160.7	103	29.2	6.99	3.67	3.22	43.4	70
	⁴ / ₁₂	154.5	85.5	32.1	6.81	3.76	3.05	31.2	77
	¹⁰ / ₁₂	148.7	81	38.5	5.74	3.08	1.16	45.6	65
8	²² / ₁₁	153.5	90	28.7	7.50	3.60	3.90	33.4	122
9	²² / ₁₂								124
	² / ₁	187.2	106	8.3	8.00	5.80	2.20	155	122
10	²⁰ / ₁₁	154.8	88.9	30.4	6.65	3.19	3.47	32.1	93
11	¹² / ₁	159.6	82	27.9	7.11	5.48	1.63	51.2	88
12	¹⁶ / ₁₀	146.3	88	27.4	5.72	2.02	3.70	41.7	75
	²³ / ₁₀	146.9	90	26.6	5.75	2.43	3.32	43	60
13	²¹ / ₁₀	156.8	89	26.6	6.02	4.17	1.85	35.6	109
	¹⁹ / ₁₁	151	83.5	26.6	5.64	3.72	1.94	45.6	99
14	² / ₁₂	145.9	92.5	25.7	5.27	2.91	2.36	32.6	65

¹ urea.

7. Jr. No. 5429. Woman aged 75. Hypertension for several years. ⁷/₁₁ 1946: Increasing difficulty in swallowing. Feverish. Admitted ⁹/₁₁. B. P. 200/90. Slight edema. Considerable paresis of gullet, with difficulty in taking food (regarded as bulbar paralysis due to hemorrhage).

le.

Erythrocytes in million	Hematocrit	Solids %	Remarks
3.97	35		Apoplexia cerebri.
5.31	35		Hemiplegia. Comatose from $\frac{20}{10}$. Died $\frac{27}{10}$.
6.12	43	10.82	
	49	11.15	
6.04	61	12.77	Apoplexia cerebri $\frac{30}{10}$, since then comatose.
5.60	62	11.59	Died $\frac{3}{11}$.
9.98	68	12.13	
5.97	64	10.65	
4.50		9.91	$\frac{21}{11}$ apoplexia cerebri. $\frac{2}{11}$: 1,500 cc saline solution intraven. Died same evening.
5.57		11.38	
5.96		11.79	$\frac{3}{1}$ apoplexia cerebri. Coma. Died $\frac{5}{1}$.
3.64	29		Diabetes. Gangraena pedis. $\frac{1}{8}$ thrombosis cerebri. Soporose, by degrees comatose. Died $\frac{3}{8}$.
3.68	30		$\frac{2}{8}$ and $\frac{3}{8}$: 1,000 cc 2.6 % NaHCO ₃ i. v.
3.53	32		
5.22	42	10.8	$\frac{10}{11}$ apoplexia cerebri. Cramps with unconsciousness of some hours' duration.
5.12	48	9.70	
3.66		9.57	$\frac{3}{11}$ paresis of gullet, feverish. From $\frac{5}{12}$ tube-fed, with copious liquid.
3.35		10.14	
3.78		8.85	
3.17		8.03	
5.76		10.55	Hypertension. Traumatic intracranial hemorrhage $\frac{22}{11}$. Comatose. Died same day.
6.47			Psychosis. Refuses food. Restless. Died $\frac{3}{1}$.
5.93			
5.49		8.81	Cardiac infarction. Cardiac insufficiency, with edema. Died $\frac{20}{11}$.
4.33		11.10	Bronchial asthma. Bronchopneumonia. Died $\frac{10}{1}$.
4.46	32	7.96	Reticulosarcoma. Ascites and severe edema.
3.77	35	8.17	Died $\frac{31}{10}$.
5.52	43	8.30	Considerable cardiac insufficiency with extensive edema. Penetrating gastric ulcer. Died $\frac{26}{11}$.
5.64	47	7.58	
3.57		7.14	Cancer ovarii with metastases. Ascites, extensive edema. Died $\frac{3}{1}$.

Feverish in the first weeks. Ingestion of liquids 200—600 cc daily per os, now and then saline solution and glucose parenterally. From $\frac{5}{12}$ permanent tube-feeding with abundance of liquid. Discharged $\frac{22}{2}$ 1947, in improving condition.

8. Jr. No. 5480. Woman aged 59. Since autumn 1945 increasing cardiac insufficiency. During stay in hospital in Jan. 1946 B. P. 170/135. Flutter. Enlarged heart, with considerable insufficiency. Again admitted $22/11$ 1946. Fell in bath, with injury to head, and became comatose with gradually appearing signs of left-sided hemiplegia. Considerable restlessness. Died in the evening of same day.

9. Jr. No. 5922. Woman aged 66. In last six weeks had hallucinations and was sometimes restless. Admitted $24/12$. Psychotic, somewhat restless, thin. Refused food during stay in hospital. Was tube-fed, but vomited most of the food supplied. $28/12$: 500 cc of saline solution + 500 cc of 5 % glucose intravenously. $31/12$: 1000 cc saline solution + 500 cc glucose intravenously. In last few days considerable dehydration. Had rather profuse hemorrhage per anum. Died $3/1$ 1947. Autopsy: No pathological features of importance.

10. Jr. No. 5431. Woman aged 80. Three weeks before admission rather suddenly showed signs of cardiac insufficiency, with some precordial pains. Admitted $19/11$ 1946. Extensive edema. B. P. 195/95. Enlarged heart. Electrocardiogram: left preponderance, no certain signs of previous infarction, but yet it was assumed that she had a cardiac infarction. $20/11$: Sudden collapse and death.

11. Jr. No. 266. Woman aged 71. For 20 years troubled by asthmatic bronchitis. Has previously been in hospital on that account. Since January several attacks of asthma. Almost unconscious for a couple of days. On day before admission showed symptoms of pneumonia. Was very ill and became comatose in the course of the day. $15/1$: Some improvement. Fever with temp. up to 40° C. Treated with penicillin. Diagnosis: Bronchopneumonia. Seemed somewhat dehydrated. Ingestion of liquids per os, $15/1$: 1350 cc, $16/1$: 900 cc, $17/1$: 500 cc, $18/1$: 250 cc. On $16/1$ she was also given 1000 cc of 5 % glucose intravenously. Died $18/1$.

12. Jr. No. 4579. Man aged 62. From beginning of 1946 general glandular swellings. Biopsy showed lymphosarcoma. Several times in hospital. Gradually increasing cachexia. Last admission $30/9$ 1946. Had infiltration in both lungs and in mediastinal glands. Ascites and severe edema of the lower extremities and pronounced venous stasis. Had had several series of X-ray treatment. Died $31/10$ in cachectic state.

13. Jr. No. 4896. Man aged 52. Increasing cardiac insufficiency from autumn 1945. Angina pectoris. Considerable dyspepsia for a long time. Gastric ulcer. Last admission $18/10$ 1946. Had severe cardiac insufficiency with Cheyne-Stokes respiration. Extensive edema. Died $26/11$. Autopsy revealed a penetrating gastric ulcer. Heart considerably enlarged, with marked coronary sclerosis and signs of an old infarction.

14. Jr. No. 5622. Woman aged 47. Since August 1946, abdominal pains, emaciation and increasing cachexia. Admitted $30/11$ 1946. Had palpable tumours in epigastrium and fossa Douglasi. Considerable ascites and edema. Virchow's gland. Was treated with mercury-diuretics. Liquids per os from $4/12$ till $22/12$: 400—900 cc. Afebrile. Died $8/1$ 1947 in cachectic state. Autopsy revealed cancer ovarii with diffused metastases.

Summary.

In several patients there was noted shortly before death a considerable increase of the total bases in serum, up to 184 m. eq./L. The content of chloride was practically normal. Together with this rise there was constantly found considerable hemoconcentration and an increase of N. P. N., as well as pathological acids in the serum.

The phenomenon was noted especially in patients dying of apoplexy, but also in one who died from a different cause. In fatal illnesses attended by considerable edema the total bases were normal or reduced and neither was there any hemoconcentration or increase of N. P. N. Probably because the large supply of extracellular fluid prevents dehydration.

It is supposed that the cause of these changes is dehydration with selective loss of water. In such patients, usually highly febrile, the insensible loss of water will be considerable and parenteral administration of liquid is in most cases omitted. One patient, in whom these electrolytic changes might have been expected, was given copious injections of liquid containing sodium bicarbonate. There was found no dehydration and likewise no increase of the total bases, in spite of copious administration of Na. On account of the dehydration the renal filtration probably becomes very much reduced so that the functioning of the kidneys as apparatus for regulation of the electrolytes becomes greatly restricted.

Methods. The total bases have been determined electrolytically by means of Herman Nielsen's apparatus. Chloride and alkali-reserve by Van Slyke's and the serum proteins by Howe's method.

References.

- Allot, E. N.: The Lancet, 236: 1035: 1932. — Atchley, D. W. and Benedict, E. M.: Journ. Biol. Chem. 73: 1: 1927. — Broch, O. J.: Acta Med. Scand. Suppl. 166: 1945. — Broch, O. J.: Ibidem, 125: 139: 1945. — Fabricius Hansen, I.: Undersøgelser over den agonal Acidose: Disp. København 1947. — Iversen, P. and Hansborg, H.: Acta Med. Scand. 57: 95: 1923. — Peters, J. P., Wakeman, A. M., Eisenman, A. J. and Lee, C.: Journ. Clin. Invest. 6: 516: 1929. — Salvesen, H. A.: Acta Med. Scand. 69: 125: 1928.
-

(From the Medical and Surgical Departments of the Frederiksborg County Hospital, Denmark. Chiefs: Senior Physician Torben Andersen, M. D. and Senior Surgeon Carl Aaberg, M. D.)

Nephrectomy in Hypertension and Unilateral Renal Disorder.

A Survey and two Cases; one resulting in decreasing Blood-pressure but persisting Tendency to Angiospastic Encephalopathy.

By

POUL EFFERSØE.

Copenhagen.

(Submitted for publication October 2, 1947.)

In his classical experiments Goldblatt (15) has shown that a transient hypertension can be produced in dogs by placing a clamp on one renal artery. Later, a *permanent* hypertension has been successfully produced in other species of animals by interfering only with the passage of blood through one kidney. Experience from the human clinical work seems to indicate that also human beings suffering from a unilateral renal disorder may develop a hypertension of renal causation. Hayward (19) performed nephropexy in a patient suffering from nephroptosis. Before the operation the blood-pressure was 140/75 mm Hg, but about one month afterwards the patient had symptoms of hypertension and a systolic blood-pressure of 180 to 210 mm Hg, the diastolic pressure being 100 to 110 mm Hg. Six months after the first operation the kidney previously operated on was removed with an excellent subjective result and a fall of the blood-pressure to 120/58, »which it has not exceeded since then». Nothing is stated about the duration of the postoperative period of observation. At

the second operation the vessels in the stalk of the kidney were found to be enveloped in dense fibrous tissue. This example thus shows that a unilateral renal disorder of vascular causation in man may give rise to hypertension.

The pathogenesis of renal hypertension has been dealt with in detail by Scandinavian authors, among others by Ask-Upmark (1), Bing (3, 4, 5), Euler (12, 13), Hilden (20) and Holten (22).

The present view is briefly that when the blood-supply of the kidney is interfered with, there will be released from the cortex a substance (renin) of an enzymatic nature which, by its action on one of the normal globulins of the blood (hypertensinogen = renin activator = pre-angiotonin), forms a substance (hypertensin = angiotonin) which causes an increase of the blood-pressure via a universal contraction of the arterioles. This will also be brought about in the other, perhaps unaffected, kidney and may perhaps cause the release of pressor substance from this kidney too.

It has been shown by Halpert & Grollman (17) that in case of experimental hypertension of long duration pathologic changes occur in the arterioles of the kidney in the form of hyalinization and sclerosis. This alone was able to keep up the release of pressor substance, also after the factor originally starting the release had been eliminated, possibly by nephrectomy.

In 1937 Butler (8) was the first to report two cases of unilateral chronic pyelonephritis with hypertension that were cured by means of nephrectomy. Since then some two hundred cases of nephrectomy in patients with hypertension and unilateral renal disorder have been reported. In some cases a dramatic and lasting recovery has been seen but, on the whole, the results are not encouraging, which has caused a few authors, such as Crabtree & Chaset (10) and Friedman, Moschkowitz & Marrus (14), to adopt the view that only the renal disorder in itself, and not a possible hypertension, may indicate nephrectomy. The reports are many and diverse in character because no author has more than a few cases of his own to report. The existing literature contains three main problems. Until they have been better elucidated every case in which nephrectomy has been attempted must be published, irrespective of the result.

(1) How Often are Hypertension and Unilateral Renal Disorder Present in the Same Patient?

This question is of importance when we consider whether it is reasonable to submit all patients with hypertension of unknown causation to the cumbrous and expensive examinations required to reveal a possible renal etiology of the hypertension. In answering the question we may choose either to examine how many patients with unilateral renal disorder have hypertension, or to examine how frequently unilateral renal disorder occurs in patients with hypertension.

The latter procedure was chosen by Braasch (6) who found about 100 cases of unilateral renal disorder among 4,000 patients with hypertension, *i. e.* a few per cent. Somewhat higher figures were found by Ratliff, Nesbit, Plumb & Bohne (31) who found 113 cases of unilateral renal disorder among 2,055 patients with hypertension.

The frequency of unilateral renal disorders giving rise to hypertension has been examined, among others, by Braasch, Walters & Hammer (7) who examined two groups of about 1,000 patients, one group comprising patients with surgical renal disorders and the other group patients without such disorders, and found the frequency of hypertension to be higher in the group with surgical renal disorders than in the group with no such disorders. When comparing the two groups the authors considered the normal increase of the frequency of hypertension with advancing age.

In 5,000 autopsies Oppenheimer, Klemperer & Moschkowitz (26) found a total frequency of hypertension of 24 per cent., whilst in the 97 cases in which a unilateral renal disorder was found at the post-mortem examination the frequency of hypertension was 40 per cent.

In 150 patients suffering from unilateral renal disorder Crabtree & Chaset (10), on the other hand, found a hypertension frequency (14 out of the 150) that was a little less than the one found in a material of controls with the same age incidence.

Apart from the last-mentioned work, the literature, however, seems to establish that there is a connection between hypertension and unilateral renal disorder in man, but this combination is present only in a very small number of cases of hypertension, probably only in a few per cent.

(2) Results of Treatment with Nephrectomy.

The duration of the postoperative observation varies much in the published cases. This is of decisive importance, as a fall of the blood-pressure that is only transient is frequently seen immediately after the operation and, as the relapse may occur so late as up to a couple of years after the nephrectomy, several authors justly require a time of observation of 1 or 2 years. The transient fall of the blood-pressure may presumably be associated with the quiet regimen to which the patient is submitted in connection with the operation. Volini & Flaxman (35) thus found a subjective improvement and falling blood-pressure in hypertonics who were submitted also to extrarenal operations, such as hysterectomy and cholecystectomy. Ratliff & Conger (30) adopt the somewhat doubtful view that they consider the patients improved if their blood-pressures remain constant for a long time after the operation and do not exceed the preoperative level, maintaining that, normally, a hypertension will progress.

In 1944 Sensenbach (33) collected from the American literature 75 cases of nephrectomy to relieve hypertension. 7 per cent. (5) had a normal blood-pressure after a period of observation of 2 years; 25 per cent. (19) had a normal blood-pressure with a period of observation from a few weeks up to nearly 2 years; 31 per cent. (23) had a decreased but not normal blood-pressure. In 37 per cent. (28) there was no fall in the blood-pressure. This means that if none of the 19 patients whose period of observation was too short get a relapse, we may reckon with one-third recovered, one-third improved and one-third in whom the condition is unchanged or aggravated. In one out of his own 4 cases the effect was good (period of observation: $3\frac{1}{2}$ years). Similar results were obtained by Ratliff, Nesbit, Plumb & Bohne (31) and by Ratliff & Conger (30). On the other hand, Friedman, Moschkowitz & Marrus (14) found a distinct fall in the blood-pressure in two only out of 29 patients.

In the Scandinavian literature a few cases of nephrectomy, several of them with good results, have been reported by Dedichen (12), Hammerström (18), Holten (22), Kirstein (23), Lange (24), Movin, Sæborg Ohlsen & Milholt Pedersen (25), Raaschou (29) and Storm Mathisen (34). As each of these authors has a couple of cases at the utmost and as there is a general tendency

only to publish case records with good results, the unsuccessful cases being left out, the formation of an estimate of the total result based on this series of single cases would result in a wrong picture. The same applies to a certain degree to Sensenbach's material, but his figures fall in well with the fact that Patton, Page & Ogden (28) in experiments on rats, in which hypertension had been produced by means of a ligature round the renal artery, found that 20 to 30 per cent. recovered after nephrectomy.

(3) Indications.

The last-mentioned authors have shown that the duration, and not the degree, of the hypertension in rats played a part in the result of the nephrectomy. The best effect was obtained when the duration was less than 10 weeks. In a calculation at a somewhat rough estimate, the justification of which is, to say no worse, debatable, the said authors consider this to correspond to 5 years in human beings.

Schroeder & Fish (32) establish the following indications:

(A) *The hypertension must be recent (estimated at less than 2 years).*

Cases with recovery have, however, been seen after hypertension of far longer standing; Raaschou (29) thus has a case with preceding symptoms of hypertension for at least 12 years.

(B) *The disorder must be localized to one kidney and must have caused its function to decrease.*

(C) *The total function of both kidneys must be within normal limits.*

The requirement that the remaining kidney must be entirely unaffected seems justifiable as a universal rule, but cases may occur (Dedichen (11)) in which the patient is far less disabled with one affected but adequately functioning kidney than with two kidneys and considerable symptoms of hypertension. Under practical conditions it is extremely difficult to make sure that the remaining kidney is unaffected. And especially as far as cases of pyelonephritis are concerned, this difficulty may presumably cause the nephrectomy to be ineffective. But it seems to be unreasonable to require a decreased function of the kidney that is to be removed. Hypertension is so serious a disease that we must be ready to sacrifice a kidney with a normal output of urine if

it is hypertensinogenic. Moreover many clinicians have experienced that it is no use removing a functionless kidney. This has been finely demonstrated in experiments by Patton, Page & Ogden (28) who, in the experiments on rats mentioned above, only found the nephrectomy to be effective in case of total occlusion of the renal artery if the inferior pole of the kidney was still functioning because it was supplied with blood from the vessels of the ureter.

(D) *Retinitis is a contraindication and the retinal changes must be minimal.*

A marked retinitis is, however, not always a contraindication, as hemorrhages and cotton-wool-like exudates may be seen as transitory phenomena (Goldring & Chasis (16)). Castleman & Smithwick (9) have moreover shown by means of kidney biopsies on human beings that there is a close parallelism between the changes in the renal vessels and the retinal changes, which can thus be taken as a measure of the vascular changes in the entire organism.

(E) *The hypertension must be permanent.*

Schroeder & Fish do not, on the other hand, mention that it is of importance when deciding on indications that not all unilateral renal disorders possibly may give rise to hypertension. It is true that Friedman, Moschkowitz & Marrus (14) could not find any connection between the nature of the renal disorder and the frequency of hypertension, but Braasch (6) believes that calculi and hydro-nephrosis cannot *per se* make a kidney hypertensinogenic, but that a secondary »vascular imbalance» is required. In hydronephrosis he finds no relation between the frequency of hypertension and the size of the pelvis, which he considers as a measure of »back pressure». Braasch, Walters & Hammer (7) more frequently found hypertension in patients with atrophic pyelonephritis than in patients with other renal disorders. 46 per cent. of the former were suffering from hypertension as compared to 18 per cent. of the whole material of 315 patients with surgical renal disorders. In patients with urolithiasis there were 22.5 per cent. with hypertension in case of accompanying manifest infection, whilst hypertension was present in 5.7 per cent. only if they had no or just a slight infection. Moreover, they found that the frequency of hypertension in patients with atrophic pyelonephritis increased with the degree of the atrophy. The effect of nephrectomy on the hypertension was better in atrophic pyelonephritis than in the other unilateral renal disorders.

A good result of nephrectomy should thus chiefly be expected in the cases in which the patient has a unilateral infection in the urinary tract, whereas the effect on the hypertension will presumably be doubtful in case of uncomplicated urolithiasis or hydronephrosis.

Moreover, the patients operated on should chiefly be those who are below 50 years of age; for with advancing age the chances are greater that the patient is suffering from a hypertension that is not due to the unilateral renal disorder. The comparatively greatest number of good results is also seen in children and young people.

It would be of decisive importance if we could find a useful method of ascertaining whether a kidney is hypertensinogenic. It is still uncertain whether unilateral clearance determinations can solve the problem.

Writer's cases:

In the course of the years 1943—46 the Medical Department of the Frederiksborg County Hospital at Hillerød has had three patients suffering from unilateral renal disorder and hypertension. One patient was 61 years and moreover unfit for operation owing to heart disease, for which reason his case will not be reported.

Patient No. 1 (Med. Dept. 1025/45; Surg. Dept. 1216/45). A man, aged 39 years, was admitted on July 21st 1945 to the Medical Department for heat stroke? Vertigo. Failing memory. The day before he had complained of headache, giddiness and failing memory. As he became confused next day, he was admitted. On arrival to the hospital his complaints were as stated above. His consciousness was highly blurred, he was almost soporose, gave uncertain answers, was sluggish as to course of thinking and movements and was unable to remember where he lived or worked. He stated that he had always been in good health previously and he denied in particular that he should have had similar attacks previously, which was confirmed by his relatives.

Apart from increased blood-pressure (see Figure), nothing abnormal was found on objective examination.

Laboratory examinations: Hemoglobin percentage: 100—105 (Sicca); erythrocytes: 4.35 to 5.28 mill. per cmm; leukocytes: 5,760 per cmm; sedimentation rate (Westergren): 3 to 5 mm in one hour; blood urea: 20 to 34 mg% (van Slyke); urine: Normal (especially no proteinuria). Microscopic examination of passed urine and of catheter urine, and cultivation from the latter showed normal conditions. In one sediment count (Addis) 13.5 mill. erythrocytes were found, and no cylinders. Addis concentration test: Specific gravity: 1.020. Urea clearance: 45 per cent. of 54 ml (standard clearance). Wassermann's reaction in



Case 1. Blood pressure.

blood and spinal fluid, electrocardiogram, X-raying of heart and lungs, and spinal fluid: Normal.

Examination by oculist (H. Skydsgaard, M. D.) 5 days after admission: In a few parts typical compression phenomena are seen at the points of crossing of the vessels; otherwise normal conditions. Diagnosis: Hypertensio a. centralis retinae.

Examination by neurologist (H. Lind, M. D.) 9 days after admission: No focal symptoms.

X-raying of urinary tracts showed an oval calcium shadow, 2 cm long, in the left kidney. After injection of hippodine no excretion from the left kidney; otherwise normal conditions.

Cystoscopy (Surg. Dept.): When inserted for 25 cm in the left ureter, the catheter is stopped by a soft obstruction, and no urine appears. No contrast medium could be injected in direct pyelography. Otherwise normal conditions.

During his stay in the department the patient became clear but had persisting headaches of varying intensity. In the hope that his hypertension might be due to the unilateral renal disorder he was transferred to the Surgical Department for operation.

On Aug. 15th 1945 the patient was operated on by the senior surgeon Dr. Aaberg, and, as the kidney was found to be pyonephrotic, it was removed. The operation and the postoperative course were uncomplicated.

Histologic examination of the kidney:

$12\frac{1}{2} \times 7\frac{1}{2} \times 3$ cm. At the junction between the pelvis and the ureter a rather firmly fixed concrement, measuring $2 \times 1 \times \frac{3}{4}$ cm, is found. The pelvis is highly dilated; the wall is pale, thickened and displays inflammatory infiltration with diffuse hemorrhage. The paren-

chyma is narrowed, in particular the pyramids. Towards the surface, groups of small abscesses are present.

Scattered throughout the renal tissue there are areas with accumulation of leukocytes, round-cell infiltration and strong hemorrhage, also some hyalinization of the glomeruli. No connective tissue proliferation. There is slight dilatation of the capsules of the glomeruli and of the tubuli.

There is a moderate proliferation of the intima of the medium-sized vascular branches, where the latter are situated near the inflammatory foci.

No signs of tuberculosis or of the formation of any malignant tumour.
Diagnoses:

Nephrolithiasis.

Hydronephrosis med.-mag.gr.

Nephritis subacuta-subchr. dispers. hemorrhagica-purulenta mag. gr. (pyelonephritis-type).

Pyelitis subchr. hemorrhagica med.-mag.gr.

On Aug. 25th 1945 the patient was transferred again to the Medical Department. He was now feeling well; there were no headaches or giddiness. On objective examination he seemed to be more clear and less sluggish.

Examinations: Blood-pressure (see the figure). Ordinary examination of urine, microscopic examination of urine, and blood-urea showed normal conditions. Concentration test: Specific gravity 1.024. Maximum urea clearance: 60 per cent. of 75 ml.

Examination by oculist 17 days after the operation: The right papilla protruding by two diopters; it is distinctly streaked. Below the papilla a streaked hemorrhage is observed in the retinal layer of nerve fibres. The veins are somewhat bulging, but not unquestionably choked; the macular vessels slightly twisted. There is a marked compression of the veins at the points of crossing. No exudates. The left papilla is like the right one. The veins slightly choked, the vessels otherwise as in the right eye. No exudates.

Oedema papillae nervi optici o. u. Retinopathia hypertensiva. Obs.
(V. Clemmesen.)

The patient was first admitted for control 4 months after, and, for the second time, 1 year and 4 months after the operation. He had begun working again and had no headaches or approaches at angiospastic encephalopathy.

One year and ten months after the nephrectomy the patient was re-admitted because, after giddiness and headache for a few days, he had fainted while working. He was at once taken by ambulance to the hospital. On his arrival here the patient was quite clear. His blood-pressure was 170 mm Hg systolic and 115 mm Hg diastolic and during the days that followed, it was about 140 mm Hg and 90 mm Hg respectively.

Examinations: Blood-pressure, see the figure. Ordinary examination of urine, microscopic examination of urine, Addis' sediment count,

blood-urea and electrocardiogram showed normal conditions each time the patient was admitted after the operation. Intravenous pyelography showed normal conditions in the left kidney.

Examination by oculist:

4 months after operation: Only slightly irregular calibers and a slight compression at one point of crossing. Otherwise normal conditions. The ophthalmoscopic changes are thus insignificant and can hardly be termed actually hypertensive. The changes described when the patient was last admitted are, at any rate, distinctly abating.

(H. Skydsgaard.)

1 year and 4 months after operation: The arteries are of a somewhat irregular caliber, there are slight refraction phenomena; otherwise natural conditions. (Bardram).

1 year and 10 months after operation (on the day when last admitted): The vessels somewhat tortuous, as previously, of slightly irregular calibre and with slight compression phenomena. No hemorrhages, exudates or oedemata.

Hypertensio art. centr. retinae (K. K. Dreisler).

Examination by neurologist 1 year and 10 months after operation:
Diagnosis: Encephalopatia hypertensiva (Henry Lind).

Epicrisis: — In a man, aged 39 years, suffering from hypertension (about 180/115) and angiospastic encephalopathy very marked retinal changes were found in ophthalmoscopy, and examination of the urinary tracts revealed a stone at the junction between the pelvis and the ureter. At nephrectomy the kidney was found to be highly changed. Histologic diagnosis: Pyelonephritis.

During a period of observation of 1 year and 10 months the blood-pressure then remained at the upper limit of the normal, whilst at the same time the ophthalmoscopic changes had almost subsided and the patient felt subjective improvement and was fit for work. 1 year and 10 months after the nephrectomy the patient got a transient increase of blood-pressure till 170/115 mm Hg and a fainting fit which must be regarded as a mild attack of angiospastic encephalopathy.

Case No. 2: — (Med. Dept. 225/43; Surg. Dept. 398/43). A woman, aged 53 years, was admitted for pyonephrosis dx. She has been mostly in good health previously, apart from repeated occurrence of dysuria. A few months before admission her blood-pressure was 200—190 mm Hg systolic and 120—110 mm Hg diastolic while she was confined to bed.

Two months before admission the patient got a sensation of heaviness in her right lumbar region, her temperature was 38° to 40° C. and the

urine was cloudy and malodorous. In the course of the last few weeks before admission the urine became clear after administration of disinfectants of the urinary tract.

Pathological findings during stay in hospital: — Blood-pressure: 180/115, later on constant at 160/110. Sedimentation rate: 47—45 mm in 1 hour. Urine: + gelatinization with potassium hydroxide; microscopical examination of urine (passed and catheter urine): On repeated examinations numerous leukocytes, some epithelial cells, a few leukocyte cylinders, no erythrocytes. Cultivation: Growth of Gram-negative lactose-fermenting rods. Concentration test: Specific gravity: 1.020. Urea maximum clearance: 68 per cent. of 75 ml. Blood urea: 42—44—38 mg%.

X-raying: After intravenous injection of perabrodil an abundant secretion is observed from both kidneys. The right kidney is displaced in the distal direction, and the right calices and pelvis are moderately dilated. There are some small calcium shadows in the central calices and a concrement, barely the size of a date kernel, at the junction between the pelvis and the ureter. The right ureter passes off from the side of the pelvis.

Cystoscopy: The bladder is normal. A catheter is easily passed through the entire right ureter, but no urine escapes. After injection of hippodine through the catheter the renal cavities are not filled.

The patient was transferred to the Surgical Department where, owing to the infection, the concretion-filled kidney is nephrectomized.

Microscopic examination shows chronic pyelonephritis.

After the operation there was a fall of short duration in the blood-pressure to 125/65 12 days after the operation, but on subsequent control examinations three months and, later, one year after the operation the blood-pressure was 200/115 and 230/130, respectively. When last controlled, the patient stated that on three occasions after the operation she had had dysuria and fever.

Epicrisis: — A woman, aged 53 years, was admitted with pyuria and hypertension, and concretions were found in the right kidney. It was removed on a purely surgical indication. Microscopic diagnosis: Pyelonephritis. After nephrectomy there was a transient fall in the blood-pressure. During the period of observation after the operation the patient had several attacks of dysuria and fever.

Comments: — The hypertension of the first patient gave so severe cerebral symptoms that an attempt at nephrectomy was justified. The good result of this operation, in connection with the finding that a marked pyelonephritis was present in addition to the hypertension, is in conformity with the fact that Braasch, Walters & Hammer (7) found the result of nephrectomy to be best in the case of pyelonephritis. The almost complete abate-

ment of the marked retinal changes illustrates what has been stated by several authors (among others by Goldring & Chasis (16)) that even severe retinal changes may subside when the hypertension is obviated. In spite of the slight postoperative attack of encephalopathy it is unquestionable that the patient has suffered from a renal hypertension from which he recovered after nephrectomy, since the blood-pressure was higher during two weeks' confinement to bed before the operation than when the patient was admitted later.

The second patient was nephrectomized on a purely surgical indication. The postoperative fall in the blood-pressure was only transient and can be attributed solely to the quiet regimen in connection with the operation (cf. Volini & Flaxman (35)), and the relapse stresses the importance of a sufficiently long period of observation. As the patient has had dysuria after the operation, the relapse is presumably due to the fact that both kidneys have been infected; it may, however, also be due to the hypertension having persisted for so long a time that irreparable changes of the circulatory system have occurred. Lastly, the hypertension may have been of extrarenal causation.

Summary.

After a reference to the investigations of previous authors into the connection between hypertension and unilateral renal disorders, and to their attitude to the question of treatment by nephrectomy, two cases are reported.

(1) In a man, aged 39, who was admitted with acute angiospastic encephalopathy, hypertension and unilateral urolithiasis were ascertained. Nephrectomy was performed, bringing about a subjective improvement and a fall in the blood-pressure. Period of observation: 1 year and 10 months.

(2) Nephrectomy was performed in a woman, aged 53, who had a unilateral urolithiasis complicated with a vigorous infection. The blood-pressure was only changed for some time. The causes are discussed.

References.

1. Ask-Upmark, E.: *Nord. Med.* 13: 472 and 552, 1942. — 2. Barker, N. W. & W. Walters: *J. A. M. A.* 115: 912, 1940. — 3. Bing, J.: *Nord. Med.* 28: 2071, 1945. — 4. Idem: *Acta physiol. Scandinav.* 9: 276, 1945.

- 5. Idem: Lægeforeningens Aarbog 1941, Afd. 111: Klinisk Aarbog. Copenhagen 1941. P. 9. — 6. Braasch, W. F.: Canad. M. A. J. 46: 9, 1942. (Ref. Yearb. of Urol. 1942: 103.). — 7. Braasch, W. F., W. Walters & H. J. Hammer: J. A. M. A. 115: 1837, 1940. — 8. Butler, A. M.: J. Clin. investigation. 16: 889, 1937. — 9. Castleman, B. & R. H. Smithwick: J. A. M. A. 121: 1256, 1943. — 10. Crabtree, E. G. & N. Chaset: J. A. M. A. 115: 1842, 1940. — 11. Dedichen, H. G.: Tidskr. f. d. norske lægef. 65: 411, 1945. — 12. v. Euler, U. S.: Nord. Med. 18: 634, 1943. — 13. Idem: Nord. Med. 13: 498, 1942. — 14. Friedman, B. L. Moschkowitz & J. Marrus: J. Urol. 48: 5, 1942. — 15. Goldblatt, H.: Ann. Int. Med. 11: 69, 1937. — 16. Goldring, W. & H. Chasis: Hypertension. New York 1944. — 17. Halpert, B. & A. Grollman: Proc. Soc. Exp. Biol. & Med. 62: 273, 1946. — 18. Hammerström, S.: Nord. Med. 23: 1663, 1944. — 19. Hayward, W. G.: J. Urol. 51: 486, 1944. — 20. Hilden, T.: Ugeskr. f. læger. 104: 633, 1942. — 21. Holten, C.: Ugeskr. f. læger. 104: 644, 1942. — 22. Idem: Nord. Med. 11: 2119, 1941. — 23. Kirstein, L.: Nord. Med. 29: 193, 1946. — 24. Lange, J.: Nord. Med. 30: 1259, 1946. — 25. Movin, R., A. Søeborg Ohlsen & A. Milholt Pedersen: Ugeskr. f. læger. 106: 643, 1944. — 26. Oppenheimer, B. S., P. Klemperer & L. Moschkowitz: Tr. A. Am. Physicians. 54: 69, 1939 (Ref. Abeshouse, S. Surgery 9: 942). — 27. Palmer, R. S., R. Chute, N. L. Crone & B. Castleman: New England J. Med. 223: 165, 1940. (Ref. J. A. M. A. 115: 1054, 1940). — 28. Patton, H. S., E. W. Page & E. Ogden: Surg. Gynec. & Obst. 76: 493, 1943. — 29. Raaschou, F.: Nord. Med. 17: 207, 1943. — 30. Ratliff, R. K. & K. B. Conger: J. Urol. 48: 136, 1942. — 31. Ratliff, R. K., R. M. Nesbit, R. T. Plumb & W. Bohne: J. A. M. A. 133: 296, 1947. — 32. Schroeder, H. A. & G. W. Fish: Am. J. M. Sc. 199: 601, 1940. — 33. Sensenbach, W.: Arch. Int. Med. 73: 123, 1944. — 34. Storm Mathisen, H.: Tidskr. f. d. norske lægef. 65: 410, 1945.
-

From the State Bacteriological Laboratory, Stockholm,
Head: Professor Gunnar Olin,
and the Dermato-Venereological Clinic of Karolinska Institutet,
St. Göran's Hospital, Stockholm,
Head: Professor Sven Hellerström.

On the Treatment of Actinomycosis with Sulpha Drugs and Penicillin.

Report of a Successfully Treated Case¹ of Pulmonary
Actinomycosis.

By

C.-A. ADAMSON and GÖSTA HAGERMAN.

(Submitted for publication September 26, 1947.)

Older therapeutic methods applied to actinomycosis, such as x-ray treatment and administration of potassium iodide — frequently in conjunction with surgery — often proved beneficial in cases with cervico-facial involvement. Frequent improvement was also noted of the abdominal type, whereas in pulmonary actinomycosis the prognosis was found to be the worst.

Walker, in 1938, described a case of »abdominal actinomycosis» with improvement following sulphanilamide treatment. A similarly beneficial action of sulpha drugs was subsequently reported by several authors (Miller and Fell, Dorling and Eckhoff, Olgilvie, Dobson, Holman and Cutting). In Sweden, cases of this type have been described by Sandegård and Lindén.

As to »pulmonary actinomycosis», Morton (1940) seems to be the first to have observed improvement after sulpha treatment. Further therapeutic successes are on record (Stangl, Dobson, Holman and Cutting, Ladd and Bill, Constam, Fanconi, Merkle, Pillsbury and Wassersug), but in several instances merely an improvement was reported. In addition, as a rule the follow-up periods were too short to speak of actual cure of the infection.

¹ This case is published by courtesy of Professor Abraham Troell, former Head of the Surgical Department, and Docent Birger Strandell, Head of the First Medical Department, St. Göran's Hospital.

The *in-vitro* action of the various sulphanilamides upon *Actinomyces* fungi has been studied in several quarters and been found to be satisfactory by, among others, Cutting and Gebhardt (1941). As with other organisms, the resistance to sulphonamides is apparently variable, and may exceed the concentrations usually to be achieved *in vivo* (Abrahams and Miller, 1946).

The Oxford workers had already observed an *in-vitro* action of penicillin on *Actinomyces* fungi (quoted by Garrod); as has been pointed out by several authors (Christie and Garrod, 1944; Abrahams and Miller; Gerber, Shwartzman and Baehr; Dobson and Cutting), there are however considerable differences in resistance between the various strains.

Numerous cases are on record of penicillin-treated actinomycosis, satisfactory results having been obtained in instances of cervico-facial and abdominal involvement (Lyons; Herrel, Hendrikson and Lehman; Hamilton and Kirkpatrick).

Roberts, Tubbs and Bates have published two cases of pulmonary actinomycosis. One of these — previously treated unsuccessfully with various sulpha drugs, blood transfusions, and repeated surgery — seems to have been cured by two courses of penicillin, viz. 1,800,000 units administered during 5 days, and 5,600,000 units given over 4 weeks.

Gerber, Shwartzman and Baehr noted a »satisfactory result» after treatment with 14,000,000 units of penicillin given over a period of 4 weeks.

The two cases quoted are all we were able to find in the literature, in which penicillin treatment seems to have resulted in cure, and the follow-up periods are scarcely satisfactory as to duration, having regard to the notorious relapsing trend of the condition.

As far as is known to us, excepting our own only two cases of pulmonary actinomycosis have been treated with penicillin in Sweden.

The first was hospitalized at the Medical Clinic of Serafimerlasarettet and related by Dr. Ågren in the discussion at the meeting of the Swedish Society of Internal Medicine held at St. Görans Hospital in April, 1946 (not published). The patient was an elderly man, who suffered from combined pulmonary and abdominal actinomycosis. The primary result of the penicillin treatment was satisfactory, but the patient nevertheless died of a supervening adrenal process.

The second case was that of an in-patient at the Sabbatsberg Hospital and is here quoted by courtesy of Docent Crafoord. A woman in her forties had for about 5 years suffered from pulmonary actinomycosis, in the course of which gestatory aggravation had twice occurred. She was given penicillin, viz. 15,000 units at four-hour intervals for more than two months, and her symptoms disappeared. Subsequently, however, she experienced a relapse in connection with a further pregnancy.

The present case of pulmonary actinomycosis was demonstrated at the meeting mentioned above. The publication was however delayed for 18 months pending a reasonable follow-up period. (During this period a case of penicillin-treated pulmonary actinomycosis was published by Aa. Lachmann. During six weeks 4,000,000 units were given parenterally, and in the first two weeks also local penicillin treatment was used. The primary result was good.)

Case Report. A man aged 40, who previously had always been well, was suddenly taken ill, on 7. 3. 45, with marked general malaise, pain in the right side of the chest, and fever. On 8. 3. 45 he was admitted to St. Göran's Hospital. X-ray of the lungs revealed areas of increased radio-density principally in the right lower lobe, and exudation. The case was as first interpreted as one of acute pneumonia with exudation, sulphadiazine (SDi) being given for 6 days without appreciable result. The sedimentation rate, which had been 42 mm on admission, rose rapidly to about 125 mm.

A thoracocentesis was carried out on 10. 3. 45. Cytological analysis of the fluid obtained disclosed chiefly mononuclear cells. Esbach's test, 20 %₁₀₀. No growth was obtained with ordinary aerobic and anaerobic culture methods. Inoculation of the exudation into guinea-pigs failed to produce morbid changes. — The Mantoux test with 0.1 mg tuberculin was negative. On bacteriological examination of the expectoration only the common throat organisms were found. Pneumococci were not demonstrated. Inoculation of Löwenstein's medium, negative.

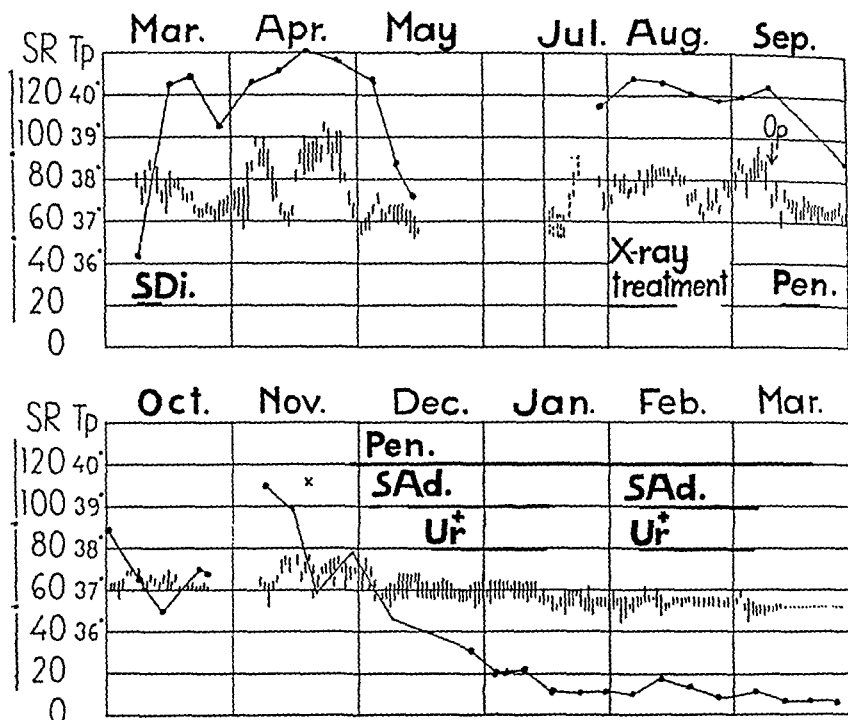
There was moderate leucocytosis, the rate keeping between 11,000 and 12,000. The cold agglutination test was negative.

For essentials of the later course of illness, see graph.

Pulmonary skiagrams taken during the months of March, April and May showed slight improvement both as to exudation and parenchymatous lesions; in the main, however, an area of increased radiodensity, the origination of which was obscure, persisted within the lower posterior portion of the right-hand inferior lobe.

General improvement took place eventually, which was also reflected by the decrease in temperature and sedimentation rate. On 15. 5. 45 the patient was discharged.

Towards the end of June, 1945 he complained of moderate pain in



Graph showing the course of illness. SR = Sedimentation rate estimated with the Westergren technique. — Towards the end of November and during the first days of December, for technical reasons only the microdetermination could be used (small dots in the curve), and the rates obtained during this period are not commensurable to the remaining.

Tp = Rectal temperature, degrees centigrade.

SDi = Sulphadiazine

Pen = Penicillin

SAd = Sulfadital

Ur⁺ = Urea

the right side of the chest. In the middle of July he once more ran a slight temperature, and on 20. 7. 45 or so a tender swelling was noted on the right side level with the 7th rib. On re-admission to the Medical Department of St. Göran's Hospital on 30. 7. 45 this swelling was slightly larger in size than a child's palm, tender to the touch and possibly fluctuating. Since in addition, there was fairly well-marked dullness in the right-hand basal area, an empyema about to perforate through the chest wall was suspected in the first place. A trial puncture failed to yield any fluid, however. A pulmonary skiagram taken on 31. 7. 47 showed no change in the condition of the lungs, but the possibility of costal osteitis was taken into consideration. The patient was given x-ray treatment from Aug. 2nd to 17th. — For a period towards the end of August, the patient experienced exceedingly severe singultus.

On 10. 9. 47 he was transferred to the Surgical Department on account of the infiltration on the right-hand side having increased in

size, the superjacent skin beginning to show discoloration, and frank fluctuation then being noted. According to the notes of the operating surgeon, «presently a cavity was entered containing a fair amount of creamy pus intermingled with gritty and granular debris». Actinomycosis was suspected as a matter of course, but bacteriological examination of the pus failed to reveal the presence of the fungus, and patho-anatomically there was nothing to suggest such an origination of the process. — The patient was nevertheless given penicillin from Sept. 13th to 23rd, viz. 200,000 units daily. — Improvement ensued, and the patient was discharged with the advice to rest for some time. On pulmonary x-ray re-examination it was found, however, that the lesions were principally unchanged.

On 7. 11. 45 the patient was again admitted, viz. in poor general condition. The incision had burst open, a sinus having developed. Actinomyces was demonstrated by anaerobic culture with material taken from the sinus tract.

The *in-vitro* resistance to penicillin was estimated at the Gonococcal Research Laboratory of the Dermatological Clinic, viz. in the following way:

Ten blood-agar plates were prepared with penicillin in concentrations ranging from 8 to 1/64 units. These plates as well as a control plate without penicillin were inoculated with the actinomyces strain over one-half of the surface, the remaining portion being inoculated with three different staphylococcal strains, the penicillin-resistance of which was known (two sensitive, one rather insensitive). The readings were made after incubation for a week under strictly anaerobic conditions.

Table 1.

Growth of Actinomyces and Staphylococcus aureus (3 different strains) on blood-agar plates prepared with varying amounts of penicillin.

PCE/ml	Actinomyces	Staphylococcus aureus		
		strain A	strain B	strain C
8	—	—	—	+
4	—	—	—	++
2	—	—	—	++
1	—	—	—	+++
1/2	—	—	—	+++
1/4	—	—	+	+++
1/8	(+)	(+)	++	+++
1/16	++	(+)	++	+++
1/32	++	++	+++	+++
1/64	++	++	+++	+++

With the procedure used here, growth of the Oxford standard staphylococcus is inhibited down to the dilutions of 1/4—1/8 unit. Hence the sensitivity of the actinomyces strain equals that of the standard staphylococcus.

The actinomyces strain proving highly sensitive to penicillin, energetic and prolonged penicillin treatment was clearly indicated.

For reasons given below, during long periods the treatment was supplemented with sulpha drugs and urea (see Table 2).

Table 2.

Synopsis of the combined treatment with penicillin, Sulfadital and urea.

Penicillin	29. 11. 45—21. 3. 46	250,000 units intramuscularly a day (106 days)
Sulfadital	3. 12. 45	14 g
»	4. 12. 45	12 »
»	5. 12. 45	2 » (discontinued on account of nausea)
»	12. 12. 45—16. 1. 46	10—8 » daily (no toxic effects worth mentioning)
»	7. 2. 46— 6. 3. 46	10 » »
Urea	18. 12. 45—16. 1. 46	24 » »
»	7. 2. 46— 6. 3. 46	24 » »
Totals, 26,300,000 units penicillin and 660 g Sulfadital.		

During the entire course of treatment daily urinalysis including examination of the sediment was carried out, without any indications whatever of renal injury emerging. The differential count was continually normal, that is to say, there were no signs of granulopenia. The leucocytic rate dropped slowly to 4,000 (on 16. 1. 46), the sulphonamide administration then being discontinued for a brief interval.

The effect of the treatment was dramatic. The general condition improved rapidly, and the temperature remained normal. The sedimentation rate was steadily decreasing. The patient gained nearly 10 kg.

The X-ray findings, which since March—April, 1945 had been practically stationary, showed considerable gradual improvement, and at discharge there was only a narrow zone of increased radiodensity in the posterior portion of the lower lobe.

On 28. 3. 46 the patient was discharged from hospital, the improvement afterwards continuing and found to be lasting.

At the time of writing, he is still in an excellent condition. For the past 18 months he has been able to do hard work in his capacity of junior surgeon on the staff of a large surgical service.

Discussion.

Pulmonary actinomycosis is a condition exceedingly hard to treat. During the protracted course of disease, enclosed foci containing pus and necrotic tissue debris are apt to develop. In the vicinity of these foci circulation is frequently very poor, *inter alia* owing to thrombus formation. In addition, penetration of chemotherapeutics into the actual foci is impeded by the fibrosis, which often occurs in the marginal zones of the process. These

factors are decidedly adverse to the action of both sulphonamides and penicillin.

Treatment having been instituted with chemotherapeutics, in auspicious cases the major proportion of *Actinomyces* elements may be expected to disintegrate after a comparatively short period of treatment. Parallel with this development clinical improvement takes place, sometimes, to proceed for a considerable length of time even though the treatment be abandoned. However, in many instances viable fungi, which can scarcely be supposed to yield to the system's own defences, will remain in the wellnigh inaccessible, encapsulated foci. Sooner or later there will be recurrences, in all probability starting from just those foci.

Thus, when dealing with pulmonary actinomycosis, the most appropriate procedure seems to be chemotherapeutic treatment continued until long after the clinical symptoms have subsided, allowing as much time as possible for maximum absorption of the pathological process (x-ray control). Not until at that stage can the conditions pertaining to circulation and diffusion be expected to have improved to a degree rendering treatment efficacious within every part of the diseased area.

From the reports quoted above it will be seen that pulmonary actinomycosis can be improved or cured by sulpha drug or penicillin treatment. On the other hand, trials with both methods combined have hitherto not been carried out as far as we were able to ascertain.

Taking into account the generally poor prognosis of the condition, in the present case an attempt to produce the most powerful action possible by combining penicillin administration with energetic sulpha treatment, was considered appropriate. Since this type of treatment necessitates large doses of sulpha drugs given over considerable periods, the sulpha-combination preparation, Sulfadital, comprising sulphathiazole, sulphadiazine, and sulphamerazine, was selected (Frisk, Hagerman, Helander, and Sjögren).

Sulfadital was given for 9 weeks in all. The daily dose was 8—10 grammes, and the total, 660 grammes. Notwithstanding this high sulpha dosage no signs whatever were observed of injury to the hemopoietic system or the kidneys.

In order further to increase the therapeutic action, and to counteract the danger of renal concrement formation, during the course of sulpha treatment also urea was given (see, *inter alia*, Wallersteiner, 1943; Frisk, and Hagerman, 1944).

Summary.

A small number of cases of pulmonary actinomycosis with improvement or cure following treatment with sulpha drugs have been described in the literature. Of later years, some few cases treated with penicillin have been recorded in addition.

The present writers give an account of a case of pulmonary actinomycosis, in which the bacteriological diagnosis could not be established until the patient had presented clinical symptoms, initially uncharacteristic and resembling those of pleuropneumonia, for 8 months.

In order to attain optimal therapeutic action, the patient was treated with penicillin, viz. 250,000 units daily given over 4 months, as well as with 8—10 grammes of Sulfadital (a sulpha-combination preparation), administered simultaneously, and with 25 grammes of urea per day.

The advantages are discussed of such a combined prolonged treatment.

Eighteen months after termination of treatment, the patient was still perfectly well, and failed to show any indications of recurrence.

References.

- Abrahams, I. and Miller, J. K.: *J. Bact.* 1946: 51: 145. — Christie, R. V. and Garrod, L. P.: *Brit. M. J.* 1944: I: 513. — Constam, G.: *Schw. Med. Wschr.* 1943: 73: 9. — Cutting, W. and Gebhardt, L. P.: *Science* 1941: 94: 568. — Dobson, L., Holman, E. and Cutting, W.: *J. Am. Med. Ass.* 1941: 116: 272. — Dobson, L. and Cutting, W.: *J. Am. Med. Ass.* 1945: 128: 856. — Dorling, G. C. and Eckhoff, N. L.: *Lancet* 1940: II: 707. — Fanconi, G.: *Schw. Med. Wschr.* 1943: 73: 175. — Frisk, A. R.: *Nord. Med.* 1944: 22: 1227. — Frisk, Hagerman, Helander, Sjögren: *Brit. Med. J.* 1947: I: 7. — Gerber, Schwartzman, and Boehr.: *J. Am. Med. Ass.* 1946: 130: 761. — Garrod, L. P.: *Brit. Med. J.* 1944: II: 528. — Hagerman, G.: *Sv. Läkartidn.* 1945: 42: 2981. — Hagerman, G.: *Nord. Med.* 1944: 24: 1944. — Hamilton, A. J. C. and Kirkpatrick, H. J. R.: *Brit. M. J.* 1945: I: 728. — Hendrikson, G. G. and Lehman, E. P.: *J. Am. Med. Ass.* 1945: 128: 438. — Herrel, W. E.: *J. Am. Med. Ass.* 1944: 125: 1003. — Herrel, W. E.: *Penicillin and other Antibiotic Agents*, Saunders 1945. — Lachman, Aa.: *Ugeskr. f. Læger* 1946: 108: 145 (ref. *Nord. Med.* 1947: 34: 1079.) — Ladd, W. E. and Bill, A. H.: *New Engl. J. Med.* 1943: 229: 748. — Lindén, O.: *Nordisk Medicin* 1942: 14: 1437. — Lyons, Ch.: *J. Am. Med. Ass.* 1943: 123: 1007. — Merkle, Ch.: *Schw. Med. Wschr.* 1943: 73:

1230. — Miller, E. and Fell, E.: J. Am. Med. Ass. 1939: 112: 731. — Morton, H. S.: Canad. M. A. J. 1940: 42: 231. — Olgilvie, W. H.: Brit. Med. J. 1940: 2: 254. — Pillsbury, N. R. and Wassersug, J. D.: New Engl. J. Med. 1944: 230: 72. — Roberts, J. E. H., Tubbs, O. S. and Bates, M.: Lancet 1945: I: 38. — Sandegård, E.: Sv. Läkartidningen 1941: 38: 1961. — Stangl, E.: Wiener Klin. Wschr. 1941: 54: 568. — Wallersteiner, W. K. S.: Nature. 1943: 151: 586. — Walker, O.: Lancet 1938: I: 1219.

From Ullevaal Hospital, IXth (Medical) Department, Oslo.
(Chief physician: H. J. Ustvedt, M. D.)

Erythema Exsudativum Multiforme.

By

HANS JACOB USTVEDT.

(Submitted for publication October 10, 1947.)

- I. The clinical picture.
- II. Relations to tuberculosis.
- III. Other etiological possibilities.

I.

The Clinical Picture.

Both in dermatological and in internal medical literature there prevails a good deal of confusion with respect to the clinical delimitation of Erythema exsudativum multiforme (E. m.). The author, whose starting-point has been the relation of this form of exanthema to tuberculosis, especially to tuberculous primary infection, has found it necessary on the basis of a large body of material to illustrate the principal features of the pathological picture before taking up for examination the special problem of the relation to tuberculosis.

Most of the investigations published in recent years respecting E. m. deal with comparatively small groups of cases. E. m. is no frequently occurring disease. For example, in the internal medical departments of the hospitals it occurs with only one-tenth of the frequency of erythema nodosum (E. n.). But E. m. is in marked degree a disease presenting many aspects, which plays a part in many fields: First of all in dermatology, nextly in internal medi-

cine, ophthalmology, tuberculosis and epidemiology. I have therefore found it necessary to assemble an extensive, many-sided material, and I have had an opportunity of dealing with all cases of the disease treated in the 10-year period 1937—1946 in the dermatological department of the Rikshospital (Professor N. Danbolt), in the Ulevaal Hospital, dermatological department (Chief Physician A. Madsen), in the three internal-medical departments of the Ullevaal Hospital (Chief Physicians R. Hatlehol, C. Müller and H. J. Ustvedt), of Drammen Hospital (Chief Physician O. Römcke) and of Vestfold Hospital (Chief Physician A. Jervell), as well as in the epidemiological department of Ullevaal Hospital (Chief Physician P. M. Holst).

The material comprises altogether 219 patients, observed in eight different departments in the course of ten years.

Historical Survey.

While erythema nodosum was clearly described by Willan as early as in 1808, it was Hebra in 1860 who through his well-known classification of the erythemas introduced the term *Erythema exsudativum multiforme*, which embraced the older designations *E. marginatum*, *papulatum*, *tuberculatum*, *iris*, *annulare* etc. Hebra pointed out that it was not here a question of true erythema, but of inflammatory changes in the skin. He did not mention the accompanying changes in the mucous membranes which were described by Bazin two years later. The older literature, especially with respect to dermatology, is very voluminous. I may here refer to Tachau (1928).

In the literature from recent years two groups of investigations may be distinguished, one of which is concerned with the severe cases attended by changes in the mucous membranes, the other with the relation to *E. nodosum*, and between these two groups there seems to be, practically speaking, no connection.

In 1916 Rendu described a syndrome consisting of affections of the mucous membrane in mouth, nose, conjunctiva, genitalia and anus, as well as varicelliform exanthema with purpura on the extremities. In 1917 Rendu and Fiessinger reported several such cases and gave to the malady the designation «ectodermose érosive plurorificielle», a name which has later to some extent been employed in the Latin form: *Ectodermosis erosiva plurorificialis*.

In 1922 the American authors Stevens and Johnson spoke of »a new eruptive fever with stomatitis and ophthalmia», which they assumed to be a hitherto undescribed form of disease. Baader in 1925 described a similar pathological picture and supplied still another name: dermatostomatitis.

The combination of exanthemas resembling erythema multiforme with symptoms from various mucous membranes has later been the subject of a long series of case-reports. I may here refer to Keil (1940) and *Report of Commission on Acute Respiratory Disease*, Fort Bragg (1946). The tendency to apply distinctive names to the diseases seems to be declining and most authors agree with Ramel (1929) in his view that these manifestations must be regarded as special forms of erythema exsudativum multiforme. From Denmark Jersild (1945) has described 25 cases with affection of mucous membranes and O. Christiansen (1946) and Tousgaard (1946) respectively 12 and 3 cases. From Norway P. Owren (1943) has reported 2 cases of considerable interest.

From certain quarters it has been proposed to include also the so-called *Behcet's syndrome* (»triple symptom complex») among the forms of E. m. Behcet's syndrome is composed of 1) transitory aphthous changes in the mucous membranes, 2) ulcerations on the genitalia and 3) affections of the eyes, especially hypopyoniritis, occasionally neuroretinitis. Several cutaneous manifestations have been observed at the same time, such as acne, pyoderma, erythema nodosum, induratum and multiforme. Especially on the basis of the affection of the eyes, however, Behcet believes that the differential diagnosis from true E. m. is clearly established.

Also in case of *ulcus vulvae acutum* (Lipschütz) there have been observed stomatitis, eye-affections and exanthemas which may be suggestive both of E. n. and E. m. (Bussalay, Tamalov). Per Rotnes has reported a case of *ulcus vulvae chronicum*, anorectal syphiloma, positive Frei test and erythema multiforme. In *Reiter's disease*, which attacks, besides the joints, also the genital mucous membrane and the conjunctiva, there may be seen affections of the skin in the form of hyperkeratosis, but exanthema resembling E. m. I have not found described.

The other group of investigations from recent years is concerned with the relation between E. multiforme and E. nodosum. Here we meet chiefly with the milder, papulovesicular cases of E. m., without simultaneous affection of mucous membranes. Hebra

regarded E. m. and E. n. as being two independent, self-contained units of disease, whereas Besnier, Kaposi, C. Boeck, Mackenzie and others believed that the two forms of exanthema were to be conceived as being different manifestations of the same syndrome. Lendon included both E. m. and E. n. in his definition of «nodal fever». Pautrier points out that the same problems arise in connection with E. m. and E. n. and he thinks that in both diseases we can distinguish between idiopathic and secondary forms.

The simultaneous occurrence of E. n. and E. m. in the same patient is well known. Per Rotnes (1936) in his 182 cases of E. n. found that it was accompanied by typical E. m. in 16 of the patients. Löfgren (1946) finds that 6 out of 174 patients had E. m. together with E. n. N. Skiöld (1945) adopts a different basis for distinguishing between the two forms of exanthema than all other authors. He regards as E. nodosum only those cases where the efflorescences disappear during typical change of colour. All cases resembling E. nodosum in which such colour changes fail to appear he assigns to the E. multiforme group. That such a distinction is not tenable is clear from, *inter alia*, Löfgren's investigations, which show that the histological picture of the nodules in E. n. is the same whether their disappearance is attended by changes of colour or not. Therefore when Skiöld finds that E. n. and E. m. behave alike in every respect, it may chiefly be because his E. m. group embraces a very large number of cases of what others would call E. n. Of his 65 cases of E. m. 60 were of the type E. tuberculatum or E. papulatum, and would presumably have been catalogued as E. n. by other investigators. In 5 cases E. annulatum was present, and only two patients showed vesicle-formation.

I shall later refer to the works that have been published respecting the relationship between E. m. and tuberculosis.

The Clinical Picture.

As regards the aspect of the *exanthema* particulars will be found in the text-books on dermatology.

As to the occurrence of *affections of mucous membranes* highly varying figures are reported, the frequency being in general placed at between 25 and 60 per cent. The affection of the mouth is for the most part described as being a stomatitis aphthosa, but it

does not seem quite clear whether we must reckon with several different types. The conjunctivitis may be catarrhal, purulent or pseudomembraneous. Edmund distinguishes between a fibrinomembraneous and a papulo-vesicular type. Corneal lesions, with subsequent blindness, are reported by American authors to occur with relative frequency, while they are only seldom mentioned by European writers. Iritis seems to be an exceptional occurrence.

Papulo-vesicular eruptions may further be seen on the genital mucous membrane (balanitis, vulvitis, urethritis, vaginitis), on the anal mucous membrane and in the vestibulum nasi. The occurrence of laryngitis, tracheitis, bronchitis and gastro-enteritis is more rarely reported.

It was early pointed out by Osler and C. Boeck that the internal organs might be attacked, with symptoms from lungs, heart or central nervous system. In the most recent years the occurrence of non-bacterial pneumonia in connection with the more severe forms of E. m. has especially been a subject of great interest (Björn Knutsen, Commission on Acute Respiratory Disease, Fort Bragg).

The general health may be quite unaffected, or it may be more or less impaired, up to the most severe degrees with septic symptoms, and sometimes with fatal issue. In most cases the exanthema lasts from two to four weeks, and not infrequently there come new eruptions at intervals of days or weeks. E. m. shows in some cases a characteristic *tendency to recurrence*, which is said to be especially notable in spring and autumn. Opinions differ as to the age and sex distribution.

Histological examination reveals edema in the cutis, dilatation of the vessels in the papillary bodies, presence of granulocytes in perivascular arrangement, often a number of eosinophile cells, afterwards lymphocytes. The process is distinctly more superficially localized than in E. nodosum.

As regards the *mortality*, some American authors in recent years, for instance Blumer (1940), state that it is high in cases with mucous membrane complications. Markham in 1944 reported 4 deaths in 5 cases of E. m. combined with primary atypical pneumonia. In most investigations no deaths, or only very few, are reported.

Regarding the *etiology* little is known. Some writers, including Keil, believe that E. m. represents an infectious disease *sui generis*. Most authors regard E. m. as a dermatological morphea which

may be due to several different infections, intoxications or allergic factors. Bacteriological investigations have for the most part given negative results. Observations made in recent years, especially in connection with primary atypical pneumonia, point to the possibility of a virus infection as cause of some of the severe cases. Inoculation experiments have hitherto yielded negative results (Edgard and Syverton, Koke, Report of Commission on Acute Respiratory Diseases, Schoening, Jersild).

The Material.

Of the 219 patients 105 were treated in the dermatological, 92 in the internal-medical and 22 in the epidemiological departments. The main body consists of 180 patients, in whom the exanthema was in every respect typical as regards the aspect and localization of the efflorescences. Eighty of these patients, *i. e.*, 45 per cent, presented at the same time one or more affections of the mucous membranes. 67 patients, or 37 per cent, had pure *E. multiforme* without symptoms from the mucous membranes. Finally, I have set apart as a separate group 33 cases (18 per cent) in which, besides a typical *E. multiforme*, there were found on the extension side of the legs many or few tender nodules resembling those seen in *E. nodosum*. In these cases the picture of *E. multiforme* was predominant, the diagnosis in the hospital was *E. multiforme* and it was only on studying the description of the exanthema that I found grounds for placing these cases in a separate group.

In addition to the 180 patients forming the main body of material comes a group of 22 patients in whom typical *E. n.* efflorescences on the extension side of the legs were combined with maculo-papular exanthemas with the localization and arrangement characteristic of *E. multiforme*, but without the typical vesicles or cockades. Likewise in these cases the hospital diagnosis has always been *E. multiforme*. On the other hand, I have not here included, as Skiöld has done, typical cases of *E. nodosum* with some superficial nodules and blotches. In all my cases the *E. multiforme* character has been predominant.

Finally, the material embraces a group of 17 cases of maculo-papular exanthema, in which *E. multiforme* was diagnosed in the hospitals, but where that diagnosis, in the author's opinion, involves a certain degree of uncertainty.

Thus we have altogether:

80 cases with mucous membrane manifestations.

67 » of frank E. m. without mucous membrane manifestations.

55 » of E. m. with separate E. n. efflorescences.

17 » in which the diagnosis is uncertain.

The significance of the material lies in the fact that it has been assembled from various special hospital units, so that it may be supposed to furnish an all-round picture. The figures are in part sufficiently large to be assigned evidential force. The weakness of the material, on the other hand, lies in the circumstance that the cases have not been described by one and the same observer and that many special examinations of importance have in some cases been omitted. Therefore the figures for the positive occurrence of various phenomena are to be taken as minimum figures. The affections of the eyes have in most cases been examined by an ophthalmic specialist and the exanthema by a dermatologist, also when the patients were being treated in internal medical departments.

The affections of mucous membranes were located as follows:

In several mucous membranes	31 cases, or 17 per cent.
In the mouth, in all	69 » » 39 » »
In the eyes in all	43 » » 24 » »
In the genitalia	19 » » 11 » »

Mucous membrane affections of mouth, eyes and genitalia were found in 12 cases, of mouth and genitalia in 7, of mouth and eyes in 12, stomatitis alone in 34 and eye affection alone in 15. Isolated affection of genital mucous membrane was not observed. Patients without exanthema are not included in this material.

The cases may be roughly classified according to the aspect of the exanthema into bullar, vesicular and papular forms, but the boundaries are not sharply defined. In all the papular cases the exanthema showed typical arrangement, mostly with cockade-formation. The material comprises 44 cases of bullar exanthema, 74 of vesicular and 62 of papular. It is often stated that the exanthema in E. m. is not accompanied by itching. In contrast to that statement it is found that a large number of patients in the present material have complained of itching, in some cases described as being intense. In some cases an urticarial component

was a prominent feature, sometimes with considerable edema in the face or on the back of the hand. One patient was treated for edema of the glottis.

Affections of mucous membranes were somewhat more frequently noted in bullar exanthema (64 per cent) than in vesicular (42 per cent) and papular (41 per cent), but the difference is not great. The mucous membrane affections are thus *by no means associated with the bullar forms alone*. Of the 80 patients with mucous membrane manifestations only 28 had bullar exanthema, while 27 had vesicular and 25 had papular forms. But where several mucous membrane affections appeared at the same time the bullar and vesicular forms were preponderant (respectively 18 and 11 as against only 2 papular).

Of the 67 patients *without* affection of the mucous membranes only 13 had bullar, while 22 had vesicular and 32 papular exanthema, and in the 33 cases where isolated *E. nodosum* efflorescences appeared at the same time only 3 patients had bullar exanthema, as against 25 with vesicular and 5 with papular forms.

From these figures it appears that bullar exanthema is to a certain degree predominant where the exanthema is accompanied by affection of several mucous membranes, whereas the bullar form is rarely seen where isolated *E. n.* efflorescences occur simultaneously with a typical *E. multiforme*. Meanwhile, it can *hardly be justifiable to establish separate, distinctly delimited groups on the basis of the aspect of the exanthema*.

In the more severe cases the exanthema was sometimes hemorrhagic, without any essential difference being otherwise observed between these cases and the others. Thus there are no grounds for setting up a separate hemorrhagic form.

Age and sex Distribution.

Figure 1 shows the percentual distribution in the different age groups. In the recurrent cases it is here reckoned with the age on admission to hospital. The curve shows a somewhat protracted maximum in the ages from 20 to 40 years. 6 per cent were under 10 years old, 18 per cent under 20 years, 20 per cent over 40. Most of the hospitals which have furnished material for this investigation do not admit children to the same extent as adults, so that the figures for the years of childhood (patients under 15 years old) may be too low.

60% -

50 -

40 -

30 -

20 -

10% -

— *E. m.*
 - - - *E. n.*
 — *Rh. ac.*

10 20 30 40 50 60 70 80
 years

Fig. 1. Age distribution: erythema multiforme, erythema nodosum, rheumatismus acutes.

For comparison is set forth the age distribution in a collection of *E. nodosum* material from Ullevaal Hospital in the years 1916—1932, embracing 314 patients (Ustvedt and Johannesen). The *E. n.* curve shows a far more accentuated maximum in the ages from 20 to 25 years, a peak which for this period concurs well with Scheel's curves for tuberculous primary infection and for exudative pleurisy. As it might be imagined that the constantly proceeding displacement of the time of tuberculous primary infection towards higher ages may have led to a similar shifting of the age distribution for *E. nodosum*, I have investigated this matter in a collection of 180 cases from the years 1937—1946. It is found that the curve here follows the same course as in the period 1916—1932. This would seem to indicate that the age curve for *E. nodosum* does not follow the movement of the infection curve, but that the tendency to react with *E. n.* to the tuberculous primary infection is in marked degree associated with the years of childhood and youth (cf. Ustvedt: »Continued Investigations on the Relation of Erythema nodosum to Tuberculosis», Nordisk Medicin 1948).

Finally, there is entered in the same graph the age distribution

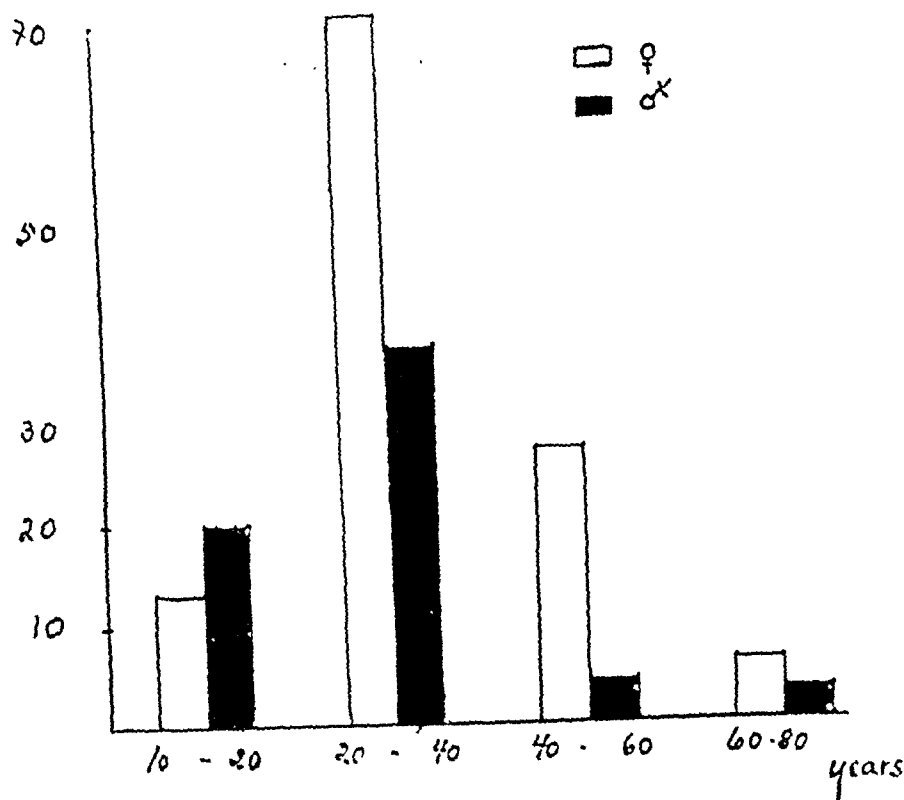


Fig. 2. Sex distribution: erythema multiforme.

in a collection of 226 cases of *rheumatic fever* from Ullevaal Hospital, Dept. IX, in the period 1925/35. Also here the figures for 1937/46 are found to give the same curve. The age distribution for *E. multiforme* appears, broadly speaking, to correspond to the findings in *rheumatic fever*, although the age maximum is reached 10 years later, while in both cases there is a striking divergence from the *E. nodosum* curve. The *sex distribution* is shown in Fig. 2, where the actual number of cases in each age group of male and female patients is given. It is noteworthy that, while the males preponderate in the age group 0—20 years (61 per cent), the females are decidedly in the majority in the other age groups. Only 7 men out of 40, *i. e.*, 18 per cent, are over 40 years old. One third of the males are under 20 years old, of the females only one tenth, while for patients over 40 years old the ratio is almost exactly the reverse. Of the total number of 180 patients 116, or 65 per cent, were females.

In this connection I may refer to the sex distribution in case

of *E. nodosum*, where we also find a certain degree of antithesis between childhood and adult age. Among patients over 15 years old Rotnes finds 5 per cent males, Löfgren finds 8.6 per cent of the male sex among 651 cases in Stockholm in 1942—44, while Mascher reports 14 per cent. In the present material of *E. m.* cases 30 per cent are men over 20 years old, that is to say, somewhat higher figures.

Among children, however, the characteristic difference between the sexes as regards the incidence of *E. n.* is not so pronounced. Rooswall in 1936 in a collection of 551 cases found that 59 per cent were girls. Comby states that among 170 cases two thirds were girls. On the other hand, no preponderance of boys, such as is seen in the present *E. m.* material, seems to have been noted in case of *E. nodosum*.

If we examine the sex distribution in the different groups of which the material is composed, we find *in the cases with mucous membrane affections about equally many females as males* (39 and 41). In the cases of frank *E. multiforme* without mucous membrane symptoms, however, the female patients constitute about two thirds (69 per cent) and in the group with *E. nod.* efflorescences together with *E. multiforme* *95 per cent are females*. The males are in the majority (59 per cent) in the group with stomatitis, while females dominate among the cases with affections of the eyes (63 per cent). In the group with ocular lesions alone 13 of the 15 patients are females. Already here we meet with a certain antithesis between mouth and eye affections, a matter to which I shall later revert.

Thus it is seen that in the group with mucous membrane affections the younger male patients predominate and that in the group with combination of *E. n.* and *E. m.* we find almost exclusively women aged from 20 to 40, while the cases with frank *E. m.* without symptoms from mucous membranes occupy an intermediate position.

The distribution of the cases among the different *months* of the year is seen from Table 1, where for comparison I have entered the monthly distribution in the *E. nodosum* material (Ustvedt and Johannesen). Both forms of exanthema show a maximum in April—June and December—January. But *E. m.* has also a maximum incidence in September, whereas *E. n.* is here at a minimum. Thus there exists some tendency to an accumulation of *E. m.* cases in the spring and autumn, but not as a

strongly dominating feature. H. Hegna in 1944 found a similar monthly curve for acute rheumatism, but with the difference that here the autumn maximum was reached in August. The figures, however, are small and do not admit of any definite conclusions.

Table 1.

Distribution throughout the year.

	<i>E. multiforme</i>	<i>E. nodosum</i>
January	150 %	112 %
February	56 "	104 "
March	87 "	90 "
April	111 "	126 "
May	111 "	126 "
June	135 "	114 "
July	95 "	90 "
August	80 "	68 "
September	127 "	68 "
October	47 "	68 "
November	87 "	100 "
December	111 "	126 "

In the literature there seems to be no record of *epidemic* occurrence of *E. multiforme*. In the present material there is found an example of simultaneous occurrence of *E. m.* in two members of a family. In the epidemiological department there were once treated two cases from the same house.

Recurrences.

In *E. multiforme* new outbreaks of exanthema and/or symptoms from mucous membranes during the course of the disease constitute a prominent feature in the clinical picture. The distinction between exacerbation in the course of the original attack of the disease and an actual recurrence will be arbitrary. In order to be permitted to speak of a recurrence I have elected to demand a symptom-free interval of at least *three months*.

In 39 out of 180 cases, or 22 per cent, one or more recurrences had taken place. Here we find a striking difference between the separate groups, seeing that 40 per cent of the cases in the group with mucous membrane symptoms were recurrent, as against 9 per cent of those with frank *E. m.* and only 2 per cent of the cases with combined *E. m.* and *E. n.* Among the cases with mucous membrane affections patients with lesions of the eyes showed

remarkably little tendency to recurrence (5 out of 26 cases), while patients with stomatitis had a stronger tendency to recurrence than the average (27 from 53). Among the recurrent cases there was a preponderance of male patients (23 against 16 females).

As regards *E. nodosum* we find greatly varying figures for the tendency to recurrence. Rotnes reports 18 per cent, Ustvedt and Johannesen 10 per cent, Löfgren likewise 10 per cent, while Skiöld finds no less than 26 per cent. Most authors agree that recurrences are seen far more frequently in non-tuberculous than in tuberculous cases of *E. nodosum*. Thus Skiöld reports 30 per cent and 7.9 per cent respectively. Edström found a tendency to recurrence in 23 out of 142 cases of *E. nodosum*, and on follow-up examination of 19 of these patients he found no cases of tuberculosis, while two had died of rheumatic infection.

Twelve patients with *E. multiforme* in our material had *previously had E. nodosum*. Where the exanthema was an unmixed *E. m.* it was found that *E. nodosum* had previously occurred in only 5 out of 147 cases, *i. e.*, in 3.5 per cent. In cases of combined *E. n.* and *E. m.* previous occurrence of *E. nod.* was noted in 7 out of 55 cases, or 12.5 per cent.

The *number* of recurrences varies considerably, and likewise the length of the symptom-free intervals. Some patients had had a few outbreaks at intervals of up to 15 years, others had had exanthema several times yearly for periods of up to 20 years. Most of the patients stated that they had had exactly the same symptoms from the mucous membranes and the same kind of eruption every time. Of the 32 cases of recurrence in the mucous membrane group, however, 5 of the patients had during several years had only aphthous stomatitis, and it was not until later that there came an eruption on the skin. But there are also seen examples of recurring exanthema where the stomatitis first appeared together with a later eruption. Only three patients had noticed that the eruptions appeared in the spring and autumn, while one stated that the spring sunshine called forth the exanthema every year. The great majority could not mention any time of predilection or any special provocative causes. The individual eruptions lasted as a rule from 8 to 14 days.

In all twelve patients who had earlier had *E. nodosum* there has been only one previous outbreak. The interval between the attacks varied up to 29 years.

Clinical Symptoms.

In a large proportion of the cases *prodromal symptoms* were observed before the exanthema appeared. In the group with mucous membrane affections prodromes occurred in one third of the cases, in the form of »symptoms of a cold», feverishness, indisposition, cough, that is to say, uncharacteristic symptoms. 3 of the 28 had vomitings, 2 had pains in the joints, 1 had dysphagia. The prodromes lasted from 2 or 3 up to 8 days, occasionally longer.

In the group with frank E. m., without symptoms from mucous membranes, the situation was about the same. Only two patients complained of pains in the joints before the exanthema appeared.

In the group with combined E. m. and E. n., on the other hand, prodromes appeared in *twice as many cases*, namely, in two thirds of the patients, and here they were of a somewhat different character: most often dysphagia, *pains in the joints*, as well as feverishness, coughing, fatigue — symptoms which are also met with in the prodromal stage of the frank E. nodosum.

The diagnoses on which the patients were admitted to hospital give a little hint respecting the clinical picture in the initial stage. A large number were admitted for acute rheumatism or rheumatic fever, some few for fever of unknown cause, rather many for erythema nodosum, some for meningitis or poliomyelitis, besides many different forms of exanthema.

As regards the relationship between exanthema and mucous membrane affection, it has here not been possible to find any characteristic *sequence* in the symptoms. The general experience seems to have been that exanthema and mucous membrane symptoms appeared at the same time. In some few cases the stomatitis came some days before the exanthema, but the reverse order of appearance has also been observed. The affection of the eyes was occasionally not noted until somewhat later on in the course of the illness. We get a distinct impression that, clinically regarded, exanthema and mucous membrane symptoms are *co-ordinate phenomena*, and that we cannot speak of exanthema as a complication in stomatitis, or *vice versa*.

Affection of the mucous membrane of the nose, often with epistaxis, was noted in 12 patients, suppurative otitis media in 3, pharyngitis in 20, laryngitis in 6, including one patient with a large laryngeal ulcer, gastritis in 3, enteritis in 1 and affection of

the anal mucous membrane in 2 patients. All these figures are certainly too low, since most of the patients have not been carefully enough examined in this respect. These affections of the mucous membranes occur mainly in the severe cases, and then usually in the combined forms.

Of quite special interest are the patients with signs of *bronchitis* or *pneumonia*. Of the 80 patients with symptoms from the mucous membranes 4 showed signs of bronchitis, 8 of pneumonia. Meanwhile, X-ray examination of the lungs during the acute stage was made only in a few cases, so that the actual number of pulmonary affections may have been considerably higher. In all the cases the pneumonia took the form of bronchopneumonia. In three patients the clinical course and the radiographic picture might be said to correspond to atypical primary pneumonia. Investigation as to cold agglutination of the erythrocytes was not made in any of these cases.

In the summer 1946 at Drammen Hospital Björn Knutsen made some very interesting observations concerning the connection between primary atypical pneumonia and *E. multiforme*. In three cases he found primary atypical pneumonia, with characteristic increased cold agglutination titer, in combination with *E. multiforme*, and in one case *E. m.* appeared in conjunction with a recurrence of the infection of the air passages. As regards two of the patients there were also recorded other cases of *p. a. p.* in the family.

Similar observations have been made in the last couple of years by American investigators (Markham, Fletcher and Harris, Commission on Ac. Respiratory Diseases, Fort Bragg). Jersild found among his 25 patients 7 cases of pneumonia and 3 of bronchitis.

I shall later revert to the importance of these cases for the understanding of the etiology of *E. multiforme*.

The findings as regards *temperature* present little of interest. We meet with afebrile, subfebrile and highly febrile cases. In case of severe bullar exanthema with mucous membrane symptoms the rise in temperature *may* be moderate, while in the maculopapular cases it may be considerable and protracted. The rise of temperature seldom lasts more than 14 days. The general conditions will, as is natural, be specially affected in patients with stomatitis.

The affection of the mouth shows all degrees of severity, from slight swelling and rubor of the mucous membrane, with some few

superficial erosions, to the severest forms with trismus, intense fetor and profuse salivation, so that it is almost impossible to inspect the mucous membrane of the mouth. We see erythematous patches, often vesicles or bullae, afterwards superficial erosions with greyish-white membranes, surrounded by a red halo. The picture corresponds in general to what is seen in aphthous stomatitis. Very often there are seen at the same time *herpetic vesicles* on the lips and around the mouth, sometimes occurring when no affection of the mouth can be observed.

Most authors regard *aphthous stomatitis* and *herpetic stomatitis* as being essentially different affections. According to Thjötta, however, the aphthous stomatitis is probably produced by the herpes simplex virus. The inoculation experiments hitherto made have furnished no grounds for supposing that E. multiforme is occasioned by that virus.

Eye affections. In the 24 cases where disorders in the eyes arose simultaneously with affection of the oral and genital mucous membrane it has always been a question of *conjunctivitis*, which has been bilateral, in most cases purulent, only seldom with distinct vesicles or bullae on the conjunctiva, sometimes with a tendency to necrosis of the palpebrae, and always resulting in complete cure without complications from the cornea. Iritis has not been found in any of the cases. Presumably these cases correspond, broadly speaking, to what Edmund calls the fibrino-membranous type.

The situation is quite different where the eye affection has appeared as the *sole manifestation* in addition to the exanthema (20 cases). Here we find 10 cases of *episcleritis*, unilateral or bilateral, and 10 cases of diffuse, catarrhal conjunctivitis. Phlyctenes were not observed in any of the cases.

I have previously mentioned that the group with pure E. m. and isolated eye affection differs in several ways from the other cases with manifestations from the mucous membranes, for example, by a great preponderance of female patients. It also displays other peculiarities, namely, remarkably frequent vesicular tuberculin reaction and strikingly high sedimentation figures. The cases with isolated eye affection seems to stand much nearer to those with combined E. m. and E. n. than do the cases with other symptoms from the mucous membranes. Probably it is chiefly the episcleritis that forms the connecting link, since this

disorder is a well-known, although relatively rare, symptom in *E. nodosum*.

The cases with episcleritis are more fully dealt with in later work. I shall here merely mention that of the 10 cases in the present material 2 were probably due to streptococcal infection, 4 to tuberculous primary infection, while 2 cases presented the picture of acute rheumatism with negative tuberculin reaction and in 2 cases we were quite without etiological data.

Transitory *proteinuria* was noted in 16 out of 180 cases and transient hematuria was observed microscopically in 2 cases. The patients with genital affections generally had pyuria. In one single case an *acute hemorrhagic nephritis* occurred. The patient was a 10-year-old boy with severe exanthema and symptoms from mucous membranes. He had hematuria, proteinuria and cylindruria, increase in non-protein nitrogen (86 mg per cent), but normal blood pressure. He had slight pains in the joints, but did not present the clinical picture of an acute polyarthritis. Hemolytic streptococci were cultivated from the fauces and from the conjunctival secretion.

Moderate *glandular swellings*, mostly of the cervical glands, were commonly found. *Swelling of the spleen* was not noted in any of the cases. In 7 cases symptoms from the *nervous system* appeared, namely, in four cases meningism with normal cerebrospinal fluid, in one case benign lymphocytary meningitis, in one case zoster and in one case polyradiculoneuritis.

One case had a *fatal issue*. The patient was a 6-year-old girl with bullar exanthema and severe symptoms from mouth, nose and eyes who died after four days' illness and in whom autopsy revealed pneumonia in the lower lobe of the left lung. Both parents had syphilis, but the Wassermann test was not applied to the child. No signs of congenital syphilis were found.

In Scandinavia a fatal result from *E. multiforme* seems to be extremely rare. Jersild had one death among 25 patients, Christiansen one among 12 severe cases.

Sedimentation Rate and Blood-Findings.

In the great majority of the cases the sedimentation rate was increased, sometimes very considerably. In 56 out of 179 patients the highest SR value noted during the illness was below 20 mm,

in 69 cases it was over 60 mm, and in 23 of these it was 100 mm or more. In the first week the SR is often seen to rise, sometimes also in the second week, even if the symptoms are subsiding. The high values of 100 mm or more were specially characteristic for the patients with combined E. m. and E. n. (11 out of 55), while only 3 out of 65 patients with mucous membrane symptoms from the mouth and the genitalia showed such high values. In the small group of 15 patients with eye affection alone 7 had a sedimentation rate of 100 mm or more, including 4 with over 120 mm.

In case of E. nodosum Rotnes found among 181 patients only 6 with SR above 100 mm, and 4 of these had combined E. m. and E. n. In his material of 185 patients with E. nod. Löfgren found an SR of 100 mm or more in 25 cases (13.5 per cent). Likewise here it is remarkable that of his 7 patients with combined E. m. and E. n. 5 had SR above 100 mm.

Even if we make reservations on account of the small figures, these findings speak towards the possibility that the combination of E. m. and E. n. entails a greater rise in the SR than E. m. or E. n. alone.

On other *examinations of the blood* there was found little of diagnostic importance. Anemia seems to be a rare occurrence even in the most severe forms of erythema. As regards the colourless blood corpuscles, 5 patients had a total number of 4,000 or less, 18 had 12,000 or more, while in 122 patients the figures lay between these values. Patients with leucopenia showed granulocytopenia, with a fall of down to 600 granulocytes per cmm in one single case. Indubitable eosinophilia (400 eosinophile granulocytes or more per cmm) was found in 19 out of 110 patients examined, and in 2 of these the count was over 1,000, the highest value being 3,300.

These different hematological findings showed no characteristic distribution among the separate groups. Two patients had thrombopenia, in both cases together with hemorrhagic exanthema.

Summary.

The material includes 219 cases of erythema exudativum multiforme. 80 presented at the same time affections of the mucous membranes, 55 showed beside a typical erythema multiforme also tender nodules on the legs resembling those seen in erythema nodosum.

It is hardly justifiable on the basis of the aspect of the exanthema to establish separate, distinctly limited groups.

The age distribution curve shows a maximum in the ages from 20 to 40 years. With regard to sex, the males preponderate in the age group 0—20 years, the females are decidedly in the majority in the other age groups. In the cases with mucous-membrane-affections there are about equally as many females as males, whereas in the group with combined *E. multiforme* and *E. nodosum* 95 % are females.

In 22 % one or more recurrences had taken place, more often in the cases with affection of mucous membranes (40 %), than in the combined group (2 %). Prodromal symptoms on the contrary were more frequently found in the latter group (2/3, mostly joint pains), than in the former (1/3).

The frequency of affections of the different mucous membranes is discussed. In 3 cases the clinical picture of a primary, atypical pneumonia was observed. The stomatitis corresponds to what is seen in aphthous stomatitis. In the cases with affections of the mucous membranes the eye affections always corresponded to catarrhal, bullous or purulent conjunctivitis, whereas in the group with combined *E. multiforme* and *E. nodosum* episcleritis was found in the half of the cases with eye affections.

Transitory proteinuria was noted in 16 cases, acute hemorrhagic nephritis in one. In 7 cases symptoms from the nervous system appeared, with one case of polyradiculoneuritis and one case of choriomeningitis.

One case had a fatal issue.

The sedimentation rate showed in 23 of 179 cases figures above 100 mm after 1 hour. The high figures were especially characteristic of the cases with combined erythema nodosum and erythema multiforme. No information of value was obtained from hematological examinations.

(St. Jans Hospital, Weert, Holland.)

Mediastinitis Anterior Chronica.

By

Dr. A. J. M. LOHMAN.

(Submitted for publication October 2, 1947.)

About one year ago Nathan and Dathe¹ described eight cases of pericarditis exsudativa in American soldiers. The illness began with a more or less serious serositis of the upper bronchial tube. In addition the patients complained of retrosternal pains and in five cases pericardial friction rub could be observed. Pericardial puncture was performed. The exudate, containing leucocytes, proved to be sterile also at a cavia-test. This pericarditis disappeared spontaneously, but substernal complaints continued for a longer or shorter period. In considering the pathogenesis of this condition they emphasize the proximity of the hilar lymph nodes to the pericardial sac. In explanation they invoke a hypersensitive response by the pericardium to an offending organism.

They do not report how this pericarditis originates, viz. whether it is hematogenic or lymphogenic; whether it develops from the heart or from the surrounding mediastinum. As this pericarditis follows upon an acute tracheobronchitis or pharyngitis (according to Corning² and Braun³ the trachea and bronchi are situated in the somewhat vaguely delimited mediastinum anterior) it is probable that, first of all, a slight inflammation of the mediastinal tissue originates via peribronchial and peritracheal lymph vessels and lymph glands, which, depending on the manner

¹ David A. Nathan and Rich. A. Dathe, Amer. Heart J. 31, 115; 1946.

² Corning, H. K., Lehrbuch der Topographischen Anatomie. 1915.

³ Braun, W., Topograf. anat. Atlas, 1924.

of spreading, causes either a mediastinitis anterior *via* lymphoglandulae sternales or a mediastinitis posterior (rare). This inflammation extends to the visceral pericardium aided by the gravitation,¹ the lift-and-force-pump action of the heart,² and the coughing and the pressing of the patient.³ Thus the retrograde transport takes place. This peri-pericarditis irritates the visceral pericard and a pericarditis exsudativa results from an excessive secretion. In the same way a mediastinitis dextra or sinistra can originate by a sidelong descent of the inflammation with a successive pleuritis mediastinalis (Corning). A hematogenic origin of the pericarditis exsudativa is improbable. It cannot be assumed that the transport takes place along the tracheo-bronchial lymph vessels, the truncus bronchomediastinalis dextra or the ductus thoracicus to the angulus venosus and along the vena subclavia and the vena cava superior to the right heart, because there are no signs of myocarditis.

Assman⁴ describes some cases of purulent mediastinitis superior, which were roentgenologically and autoptically confirmed, following an acute tracheo-bronchitis or after perforation of the tracheal wall by corpora aliena. In one case the formation of gas was observed in the mediastinum anterior as a result of contagion by putrid bacteria. Schinz⁵, too, gives some examples of such »Senkungsabscesse». v. Dehn⁶ and Lorey⁷ likewise observed some cases of mediastinitis anterior acuta and they point out the difficulties of a differential diagnosis with regard to tumors, thymus-hyperplasia etc. in the mediastinum anterior.

Nathan and Dathe put the question in their article referred to above, whether these clinically not very clear cases of pericarditis exsudativa might not be the cause of the local (or total) adhesive pericarditis which is sometimes found at autopsy (Smith and Willius⁸), whereas there was no evidence in the anamnesis either of the causative factor or of the pericarditis.

So it appears that the pericarditis and the attending mediastinitis anterior pass in many cases practically without any symptom and that only an accurate and systematic examination in

¹ David A. Nathan and Rich. A. Dathe, Amer. Heart J. 31, 115, 1946.

² Corning, H. K., Lehrbuch der Topografischen Anatomie. 1915.

³ Braun, W., Topograf. anat. Atlas, 1924.

⁴ Assman, H., Klin. Roentgendiagnostik, F. Vogel, 1934.

⁵ Schinz, H., Dtsch. Z. Chir. 179, 1929.

⁶ v. Dehn, W., Berl. Klin. Wschr. 480, 1910.

⁷ Lorey, P., 8. Roentgenkongress.

⁸ Smith, H. L., and Willius, F. A., Arch. Int. Med. 50, 410, 1932.

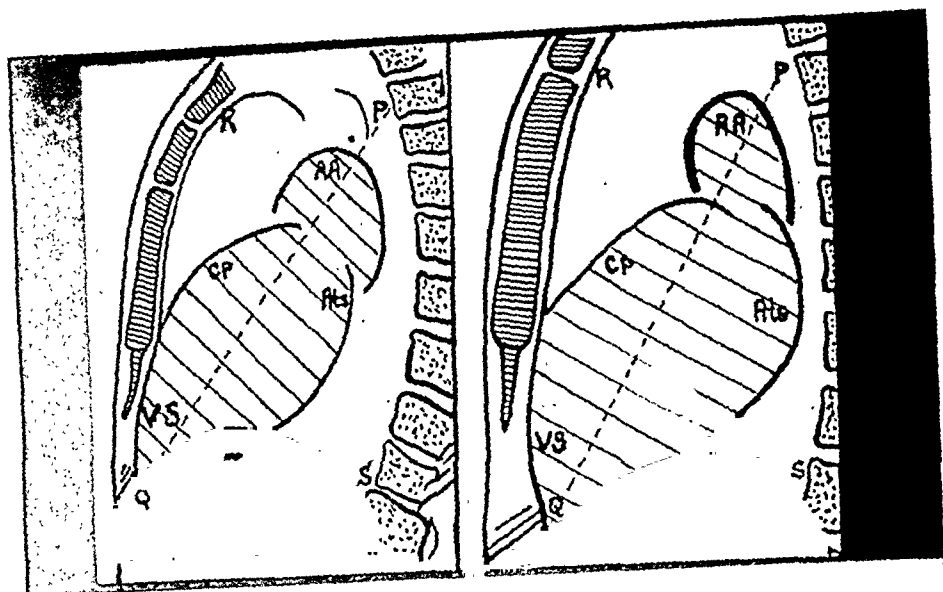


Fig. I.

a: sagittal roentgenogram of a normal man.

b: the same for a normal woman.

Heart axis VS—AA forms diagonal in trapezium PQRS.

The sinus diaphragmatica anterior is free and forms an angle $< 90^\circ$.

suspicious cases will result in the discovery of pericardial and mediastinal disturbances. Symptoms of the final remnants may be shown by kymography of the heart (Berner¹) or by tomography (Juzbašić² and Berner) or planigraphy and by cross-aimed thoracal X-ray films of the mediastinum anterior.

In the past six years we have devoted a systematic study to the mediastinum anterior. The danger of incorrect interpretation of the X-ray films proved to be considerable, the more as on account of insufficient data for comparison, a correct appreciation of the roentgenograms obtained was difficult.

After the sternum, rib cartilage and m. transversus thoracis, have been removed the mediastinum lies free. The arteriae and venae mammae intt. lie against the inner thorax wall outside the mediastinum against the costal pleura. They give blood vessels to the thymus and the fatty tissue of the mediastinum anterior. In the upper part behind the manubrium sterni the fat- and connective tissue is immediately connected to the periost. At the height of the 3rd and 4th rib cartilage the two edges of the pleural cavity are nearly connected, so that there is mostly a narrow slit. At the level of the sinus phrenico-costalis the anterior mediastinum becomes wider and forms a more or less wide retrosternal resp. xyphoidal cavity which is filled up with connective tissue.

At an aimed roentgen examination the target ray must pass through the sternum of the mediastinum anterior. We can observe:

a. Manubrium sterni, corpus sterni and processus xyphoideus run in a slightly curved line of which the connective line runs parallel to the front of Th₄—Th₅.

b. The anterior sinus diaphragmaticus forms an angle of $< 90^\circ$ between the back of the sternum resp. proc. xyphoideus and the diaphragm.

c. In deep inspiration this sinus appears to protrude far beneath the apex of the proc. xyphoideus.

d. The facies articularis of the manubrium and the corpus sterni form two parallel lines.

e. The proc. xyphoideus proceeds centrally from the sternum and extends in the direction of the corpus sterni.

f. The cavity of Holzschnecht formed by the right atrium (A. t. d.) the front of the spinal column and the diaphragm is triangular.

¹ Berner, L., Schw. med. Wschr., 12, 256, 1946.

² Juzbašić, D., Schw. med. Wschr. 12, 256, 1946.

g. The axis of the heart (Vs—AA) forms the diagonal of the quadrangle PQRS:

h. The m. transversus thoracis, the fascia endothoracica and the retrosternal connective tissue form an equally narrow border running parallel to the sternum, which is clearly marked off against the adjoining heart.

i. On the front-backward X-ray picture of the thorax the angle between the diaphragm cupola and the heart is $< 90^\circ$. The centrum tendineum is not raised. This last point is extremely important for the differential diagnosis!

In the course of six years we have had the opportunity to study five cases of mediastinitis anterior chronica more closely and we believe that the above mentioned origination viz. as a result of an acute tracheo-bronchitis or pharyngitis (a. o. influenza) as in the cases of Nathan and Dathe must be considered as being likely or certain. We could study the final results, caused by the formation of adhesions and the ensuing shrivelling, both roentgenologically and microscopically. *Said writers described the first acute phase; we observed the rest-phenomena.* As in some cases, which have thus far been recorded as »cardiac neurosis» on account of the absence of objective phenomena at the usual examinations, a special technique shows objectively perceptible anomalies, it is possible to recognize these cases of »cardiac neurosis» as organic diseases which is in the interest of patients (simulation, hysteria!).

We studied a total of five patients: 2 men and 3 women between 27 and 65 years.

In all these cases *the history proves to be characteristic.* The illness sets in acutely with the symptoms of influenza, attended by coughing, high fever and oppression on the middle of the chest. These acute symptoms soon disappear. After that, the patients keep on complaining more or less of an oppressive pain behind the sternum, not radiating to the shoulder or left arm, of being easily tired, and of increasing complaints upon exercise which disappear when they rest. After some months a »pseudo-funnel-chest» may develop, by which we mean the gradually increasing deepening of the small sac under the angulus costarum by an inward retraction of the proc. xyphoideus as a result of the shrivelling of the retro-sternal, resp. retro-xyphoidal connective tissue of the mediastinum anterior. This anomaly may be distinguished from the real funnel-chest or shoemakers-chest, such as we can

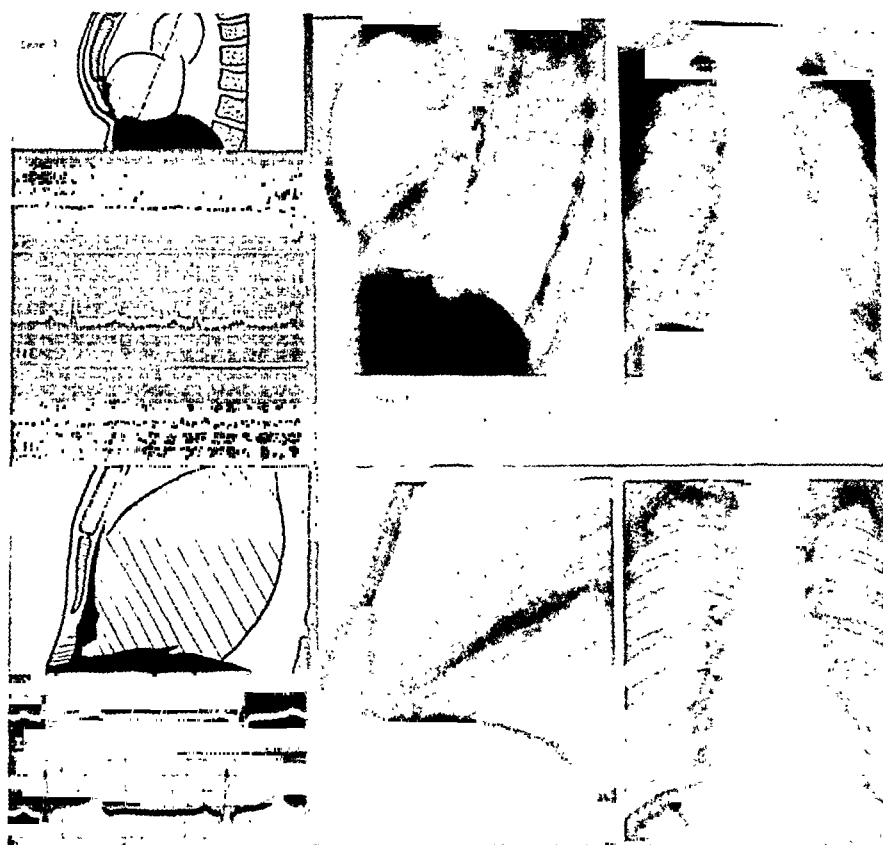


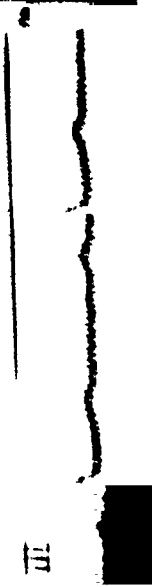
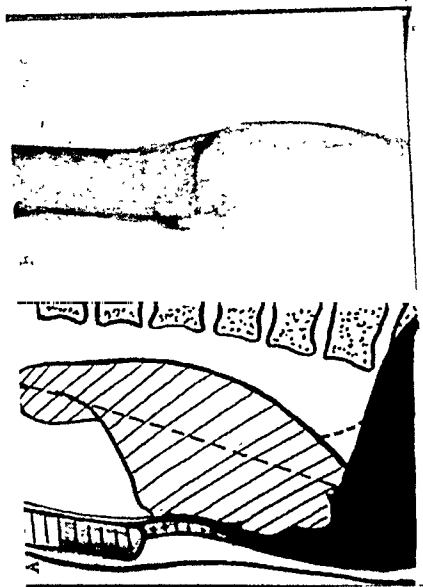
Fig. II.

Case I: 65 years old, fine »pseudo» funnel chest, adhesive thickenings behind the proc. xiphoidens. On the A.—P. Roentgenogram no anomalies.

Case II: 34 years old, thickening behind the proc. xiphoidens. Elevated sinus diaphragmaticus anterior.

see with patients with old rickets or as a symptom of the status dysrhapicus.

Case I: Woman of 65 has been complaining for 7 years of an oppressive pain behind the sternum. At the time the complaints originated after an »influenza», attended with high fever, shortness of breath and a feeling of oppression at deep respiration. When she exerts herself the complaints increase, when she rests they disappear. No cardiac pulsations, no radiation to the left arm. The heart-sounds are normal. Typical pseudo-funnel-chest acquired later on. Blood pressure 200/95; sedimentation rate 13—37 mm; reaction of v. Pirquet: negative; reaction of Wassermann: negative. Roentgenogram of the thorax (sagittal): manubrium and corpus sterni form an angle of $<180^\circ$. The facies



Case 3

Fig. III.

Case III: 42 years old, elevated sinus diaphragmaticus anterior with thickening of the retrosternal connective tissue. On the A.—P. Roentgenogram no anomalies can be seen.

articularis diverge in front. The proc. xyphoideus is drawn in. The anterior sinus diaphragmaticus is about 90° and drawn up to the proc. xyphoideus. The quadrangle PQRS has been deformed to a trapezium with the smaller side to the diaphragm. The articulation of the proc. xyphoideus has been moved backwards. The front of the triangle of Holzknacht has been overturned forwardly and runs parallel to the spinal column. The heart axis no longer forms the diagonal in the trapezium, but stands \perp on the basis; the retrosternal resp. retroxyphoidal tissue is thicker. On the frontal X-ray film of the thorax there are no changes worth mentioning. Electrocardiogram: apart from a slight notching of the QRS-complex and a high RII there were no changes to be seen.

Case II: Man, 34 years, had influenza 4 weeks ago. Since the second week he complains of a pain on the middle of the chest and in the beginning he could not breathe very well. The patient is easily tired and is worried about his heart (angina pectoris?!). Blood pressure is 130/80. The heart sounds are normal. Sedimentation rate 13—27 mm. Reaction of v. Pirquet is negative. The reaction of Wassermann is negative. Roentgenogram of the thorax (sagittal): manubrium and corpus sterni form an angle $< 180^\circ$. The facies articulares diverge in front. The anterior sinus has been raised to the proc. xyphoideus and forms an angle of 90° . At the back of the proc. xyphoideus there is a thickening of the retrosternal, resp. retro-xyphoidal tissue to be seen. Electrocardiogram: no changes worth mentioning.

Case III: Man, 42 years, had an acute tracheo-bronchitis $6\frac{1}{2}$ months ago, followed by an oppression on the middle of the chest, especially when exerting himself; even when he rests the feeling of oppression remains. He is easily tired. Pain irradiates to the scrobiculum cordis and to the right. There is no connection with breathing. Blood pressure: 135/95. Once a pericardial friction rub was observed!! The heart sounds are slightly distant. Sedimentation rate 20—38 mm. Reaction of v. Pirquet is ++! Reaction of Wassermann: negative. Roentgenogram of the thorax (sagittal): the anterior sinus has disappeared, the diaphragm has been drawn to the proc. xyphoideus which has been shifted and bent backward; on the frontal side the heart is adherent to a large extent. The heart axis stands \perp in the diaphragm. The triangle of Holzknacht is high. At the back of the proc. xyphoideus large shadows can be seen which increase downwards. The basis of the quadrangle PQRS has become smaller by the shifting of the proc. xyphoideus towards the spinal column. Electrocardiogram: no changes worth mentioning.

Case IV: Woman, 37 years, has complained for 3 years of an increasing pain and oppression on the middle of the chest, which when she exerts herself radiates to the right and to the scrobiculum cordis. At that time she had gone through acute illness attended with violent coughing. Blood pressure is 145/90. Heart sounds are normal. Sedi-

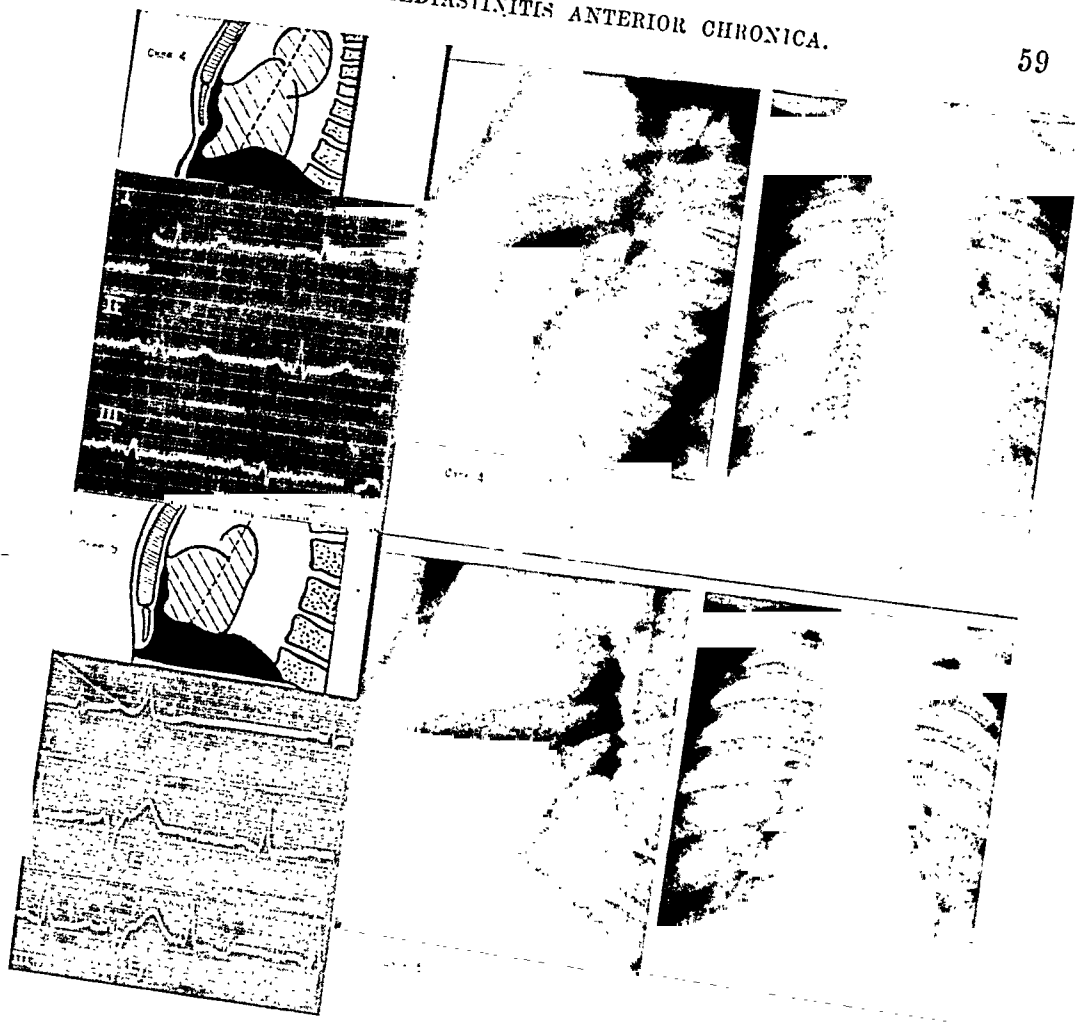


Fig. IV.

Case IV: 34 years old. Thickening behind the proc. xyphoideus. Lifting of the sinus diaphragmaticus anterior.

Case V: 53 years old, thickening behind the proc. xyphoideus. Lifting of the sinus diaphragmaticus anterior. E. C. G. shows some ventricular extrasystoles.

mentation rate 3—7 mm. Reaction of v. Pirquet is negative. Reaction of Wassermann is negative. Typical pseudo funnel chest. Roentgenogram of the thorax (sagittal): manubrium and corpus sterni form an angle of $< 180^\circ$. The proc. xyphoideus is drawn in. Retrosternally a thickening of the precordial tissue can be seen. The heart axis stands \perp in the diaphragm. The triangle of Holzkecht is tilted to a large extent. The front of the heart is adhered to a large extent. The anterior sinus diaphragmaticus has disappeared as the diaphragm has been raised towards the proc. xyphoideus. On a frontal X-ray film

of the chest no anomalies can be seen. Electrocardiogram: no changes worth mentioning.

Case V: Woman, 53 years, has complained of a pain on the middle of the chest for more than 6 months; especially at night she feels very much oppressed. She becomes easily tired. Pain does not radiate to shoulder or left arm, no connection with the respiration. Blood pressure is 160/100. Pulse is irregular on account of extrasystoly. The heart sounds are somewhat muffled, but otherwise nothing remarkable. The beginning of a funnel chest. Sedimentation rate 4—9 mm. Reaction of v. Pirquet is positive. Reaction of Wassermann negative. Roentgenogram of the thorax (sagittal): manubrium and corpus sterni form an angle of $< 180^\circ$. The proc. xyphoideus points backward. The heart axis is bent forward and almost \perp diaphragm. In front the heart adheres to a large extent to the corpus sterni. Retrosternally the shadow of the mediastinum becomes thicker downwards. On a frontal roentgenogram no anomalies can be seen. Electrocardiogram: P somewhat widened; Q absent in all three derivations, slight notching of the QRS-complex, slight deviation of ST in all three derivations; a ventricular extra systole.

Table I.

Case	Age	Sex	Anamnestic period of complaints	Localization of complaints	Connection with respiration	Soon tired	Increase of complaints at straining	Blood pressure	Funnel breast	Sedimentation rate	von Pirquet	E. O. G.
T	65	♀	7 years	middle of the chest	—	—	—	200/95	++	13—37	—	normal
G	34	♂	4 weeks	do	—	+	+	130/80	—	13—27	—	do
C	42	♂	14 weeks	do	+	++	+	135/95	+	20—38	++	do
J	37	♀	3 years	Irradiating to the right	—	—	—	145/90	++	3—7	—	do
V	53	♀	1½ year	do	—	++	+	160/100	+	4—9	+	do

The uniformity of the above mentioned case histories is striking and characteristic. Three of the five patients came to ask whether they had a heart disease. One of them had made his own diagnosis: angina pectoris (No. II)!!

In case III we thought the best course would be an *operative treatment*. The seriousness and the increase of complaints, the objective certainty of precordial deformations and the fact that from additional clinical data it might be assumed that the causative process had come to a rest (temperature, blood picture, sedimentation rate, general condition of the patient and electrocardiographic investigation) justified this intervention. On Sept. 11.

1946 the patient was operated (Brandsma). The abbreviated operation report runs as follows: Medial incision across the corpus sterni and the proc. xyphoideus. The proc. xyphoideus lies in one cm deeper level inwards and is bent in the direction of the spinal column. The 4th, 5th, 6th rib are cut on both sides. The sternum is sawed through and removed together with the proc. xyphoideus. At section the typical bayonet-shape can be seen (see fig. III).

The mediastinum anterior appears to be filled up with a very tough connective tissue $1\frac{1}{2}$ cm thick. The parietal pericardium is opened. Under some pressure too much clearly yellow liquid appears. There is no syncretio pericardii. Closure of the pericard with silk stitches. Closure of the mediastinum.

The pericardial liquid proves to be sterile at a culture test. At microscopic examination of the retrosternal tissue the following data were revealed (Wijers):

Microscopically tendinous tissue with hyalinisation of the connective fibrils and on one side a small piece of bone tissue can be seen. Between the tendinous connective tissue and the periosteum we find oedematous connective tissue with protein precipitations and a slight infiltration of polynuclears and lymphocytes. Here there is a (so called «albuminous») periostitis. No evidence of tuberculosis is found.

From the above we may conclude that in the mediastinum anterior, a chronic, non specific inflammation existed, which caused a retro-position and the inward bending of the proc. xyphoideus (reclinant position). A close study of the resected part of the sternum showed that this position must be considered as being acquired. This corresponds with the patient's statement that the pseudo-funnel chest is of a recent date. Concerning the differential diagnosis the first thing to pay attention to is the pleuritis interlobaris dextra between the mid- and under lobes. On the frontal X-ray picture of cases of chronic proliferative interlobular pleurisy usually no sufficient shadows are visible. On the sagittal photo, however, a shadowed sinus diaphragmaticus can be seen which continues, however, in an oblique line upward in the lower interlobium (see fig. V a).

Comparative examination have shown that the proc. xyphoideus can be, congenitally, placed more backward as it sometimes occurs in the status dysraphicus and syringomyelia (in which these deviations are sometimes familiar). The pseudo-funnel chest

of spreading, causes either a mediastinitis anterior *via* lymphoglandulae sternales or a mediastinitis posterior (rare). This inflammation extends to the visceral pericardium aided by the gravitation,¹ the lift-and-force-pump action of the heart,² and the coughing and the pressing of the patient.³ Thus the retrograde transport takes place. This peri-pericarditis irritates the visceral pericard and a pericarditis exsudativa results from an excessive secretion. In the same way a mediastinitis dextra or sinistra can originate by a sidelong descent of the inflammation with a successive pleuritis mediastinalis (Corning). A hematogenic origin of the pericarditis exsudativa is improbable. It cannot be assumed that the transport takes place along the tracheo-bronchial lymph vessels, the truncus bronchomediastinalis dextra or the ductus thoracicus to the angulus venosus and along the vena subclavia and the vena cava superior to the right heart, because there are no signs of myocarditis.

Assman⁴ describes some cases of purulent mediastinitis superior, which were roentgenologically and autoptically confirmed, following an acute tracheo-bronchitis or after perforation of the tracheal wall by corpora aliena. In one case the formation of gas was observed in the mediastinum anterior as a result of contagion by putrid bacteria. Schinz⁵, too, gives some examples of such »Senkungsabscesse». v. Dehn⁶ and Lorey⁷ likewise observed some cases of mediastinitis anterior acuta and they point out the difficulties of a differential diagnosis with regard to tumors, thymus-hyperplasia etc. in the mediastinum anterior.

Nathan and Dathe put the question in their article referred to above, whether these clinically not very clear cases of pericarditis exsudativa might not be the cause of the local (or total) adhesive pericarditis which is sometimes found at autopsy (Smith and Willius⁸), whereas there was no evidence in the anamnesis either of the causative factor or of the pericarditis.

So it appears that the pericarditis and the attending mediastinitis anterior pass in many cases practically without any symptom and that only an accurate and systematic examination in

¹ David A. Nathan and Rich. A. Dathe, Amer. Heart J. 31, 115, 1946.

² Corning, H. K., Lehrbuch der Topografischen Anatomie. 1915.

³ Braun, W., Topograf. anat. Atlas, 1924.

⁴ Assman, H., Klin. Roentgendiagnostik, F. Vogel, 1934.

⁵ Schinz, H., Dtsch. Z. Chir. 179, 1929.

⁶ v. Dehn, W., Berl. Klin. Wschr. 480, 1910.

⁷ Lorey, P., 8. Roentgenkongress.

⁸ Smith, H. L., and Willius, F. A., Arch. Int. Med. 50, 410, 1932.

pointed out the difficulties at the examination of the X-ray films obtained from sagittal roentgenograms of the chest. Part of the cases of »heart neurosis» in which with normal routine no changes are found, can be explained by the syndrome of mediastinitis anterior chronica.

(From Odense County and Town Hospital, Medical Department, Denmark. Chief: K. Schroeder M. D., and the Biochemical Department of the Central Laboratory, Chief: J. C. Jespersen, hospital pharmacist.)

Titration of Serum with Mercuric Chloride Especially in Liver Affections.

By

TAGE GRINSTED.

Odense.

(Submitted for publication October 2, 1947.)

In continuation of a work published in 1946 (2) an account is to be given here of the titration of serum with mercuric chloride.

In 1935 Jakobson demonstrated that sera from patients suffering from myelomatosis gave a vigorous precipitation by addition of Hayem's fluid (2 % sodium sulphate, 1 % sodium chloride and 0.25 % mercuric chloride). Later Gros (4) demonstrated that by addition of Hayem's solution to sera, first a reversible and later an irreversible precipitation of proteins occurred. A comparison with the Takata-Ara reaction which also involves protein precipitations in connection with the addition of mercuric chloride, gave a very close agreement between the two reactions in the way that the more vigorous a positive Takata-Ara was the more quickly did the precipitation occur by addition of Hayem's solution.

On basis of these observations Gros worked out his titration method: to 1 ml of serum Hayem's solution is added drop by drop for the determination of the lower limit of the reversible precipitation. The serum used is always fresh serum, as he demonstrated a quicker precipitation to occur with serum which has been standing for some hours.

By means of this titration he examined 120 Takata-Ara positive sera and found that 116 of them gave initial precipitation by the addition of from 1 to 10 drops. Of 530 Takata-Ara negative sera

the 502 showed a lower precipitation limit by the addition of abt. 2.5 ml. The remaining 28 sera, on the other hand, showed a lower precipitation limit ranging from 4 to 10 drops, but a further examination of the latter category of cases gave the result that the sera were mainly sera from patients with hepatitis, and he offered the explanation of the lower values of these sera that the changes in the sera were not yet so pronounced as to give a positive Takata-Ara reaction, yet vigorous enough to give a clear lowering of the lower precipitation limit by the titration. Later it proved in fact, that the precipitation limit rose again on the clinical improvement of the condition. Gros therefore was of the opinion that by titration of serum with Hayem's solution he had found a better, and more accurate method than the Takata-Ara method for the evaluation of damage to the liver parenchyma, and he also emphasized the simplicity of the titration method, its speedy performance and simple manner of recording the strength.

These examinations were later confirmed by Vischer (15) in 1941 and, in part, by Bjørneboe (1) in 1946.

The titration method was later modified by Stolte (13) in 1940, the sodium sulphate having been left out as unnecessary. He used $\frac{1}{2}$ ml serum (not over 3 hours old) to which is added 1 ml 0.9 % sodium chloride after which it is titrated with a 0.1 % mercuric chloride solution.

By this method the lower precipitation limit of normal sera was found to be 1.6 ml or upwards. Otherwise the results fall in line with those of Gros's titration with Hayem's solution.

T. Halström states in 1944 (7) by means of the same titration on 200 sera to have found the following values: normal 1.2—2 ml, cirrhosis of the liver 0.6—2 ml, hepatitis 0.8—1.4 ml.

By examinations carried out in 1946 the author (2) confirms the examinations made by Stolte, demonstrating a close agreement to exist between the results of this titration and the Takata-Ara reaction.

In the titrations with mercuric chloride precipitations of protein in serum are brought about by the addition of mercuric chloride in a salty solution, but it has not been established with certainty which factors are of decisive importance for the degree of the precipitations. Staub and Jezler showed that changes in the composition of the serum proteins are necessary in order to produce a positive reaction, and numerous authors have sub-

scribed hereto. Especially the albumin/globulin quotient in serum is indicated to be of importance, a positive reaction being claimed to depend on a low quotient. Gros and de Vries have, however, established that this quotient is not solely decisive for the reaction as they have both of them reported cases with a very low albumin/globulin quotient and negative Takata-Ara reaction. Several authors hold the view that also other substances may influence the reactions, as *e. g.* acetone bodies, fatty acids, heparin, lecithin and cholesterol, but this view has not been confirmed by later investigators. It must therefore be taken to have been established that the serum protein changes are of decisive importance for the outcome of the reactions.

Ucko (14) holds that qualitative changes of the albumin may influence the reactions, but conclusive proof hereof has not been established. Gros and de Vries demonstrated that certain globulin fractions are able to promote the reactions and that others are able to inhibit them. De Vries (16), therefore, on basis of several examinations, arrives at the conclusion: 1) there is a connection between the strength of the reaction and the decline in the content of albumin; 2) an increase in the globulin content is no guarantee for a positive reaction; 3) an increase of globulin with a positive reaction is due to an increase in the content of euglobulin or pseudoglobulin I; 4) globulin increases on account of pseudoglobulin II increases do not give a positive reaction, on the contrary they seem to inhibit the reaction, acting as a protective colloid for the precipitation.

In Bjerneboe's work from 1946 (1) this view seems to be substantiated in a convincing manner, first of all an only fairly good agreement being demonstrated to exist between the albumin/globulin quotient and Takata-Ara and Gros's titrations, and this is explained on the assumption that the precipitations, which are due to a combination of Hg and globulin, occur the sooner the smaller the amount of protecting colloid contained in the serum, *i. e.*, albumin and pseudoglobulin II.

Accordingly it is clear that the Takata-Ara reaction and the mercuric chloride titrations cannot be specific tests for a certain disease, but may become positive in all diseases which may bring about the above mentioned changes in the serum proteins. This is the case *e. g.* in parenchymatous liver lesions, pulmonary tuberculosis, nephritis, lymphogranuloma inguinale, myeloma and sepsis.

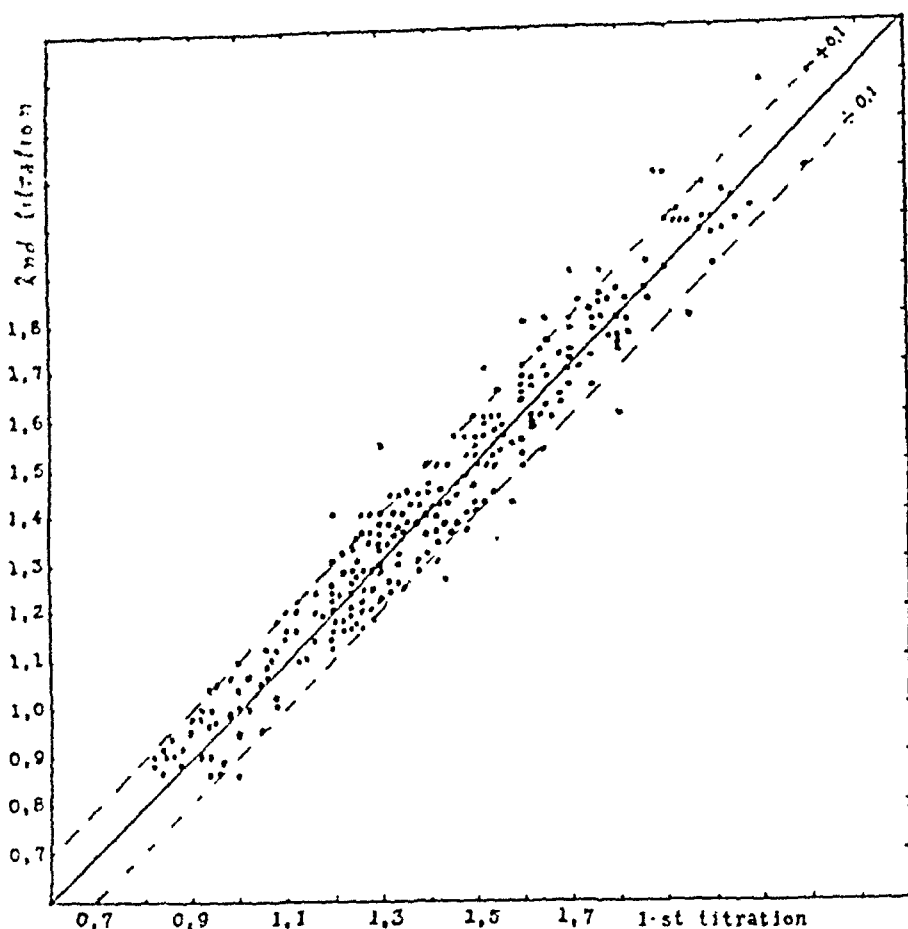


Fig. 1. 300 cases of double determinations with mercuric chloride titration of serum.

The technique employed in the present investigation is the modified Gros titration given by Stolte:

The substances used are: mercuric chloride 0.1 %, sodium chloride 0.9 %. To $\frac{1}{2}$ ml serum in an ordinary test tube (diameter abt. 15 mm) 1 ml 0.9 % sodium chloride is added. Next titration is made with the 0.1 % mercuric chloride solution (from a 2 ml pipette fixed on a stand, divided in 0.01 ml) drop after drop in quick succession until an initial reversible precipitation appears, thereafter, slowly, drop-by-drop titration until permanent turbidity. The titration value is recorded directly as the number of ml of mercuric chloride used in the titration.

In the positive reactions the precipitation sets in very suddenly so that there can be no doubt about the titration figure; in the case of the negative reactions the precipitation appears gradually,

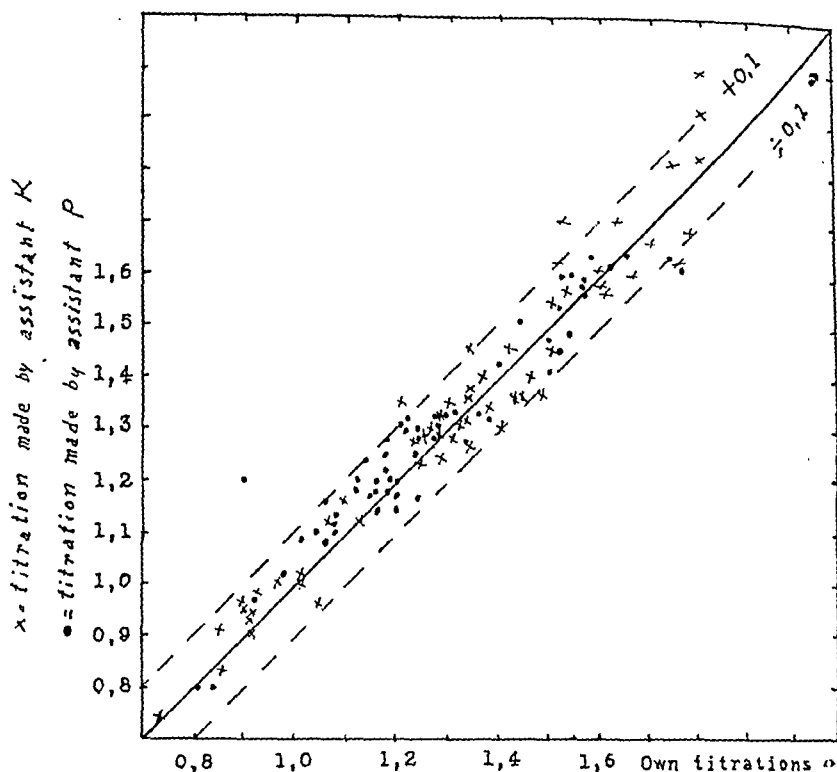


Fig. 2. 130 cases of own titrations compared with titrations made by 2 assistants.

and in the latter case it may be somewhat difficult for the inexperienced person to judge when the titration should be concluded. Consequently it may be useful to fix this limit somewhat more exactly and I have done that in the following manner: continue titration until beginning turbidity, then wait for $\frac{1}{2}$ minute and now try whether it is possible to read ordinary-sized print through the turbidity; if this is not possible, the titration is concluded; otherwise continue until this happens. With only slight experience this procedure should, however, be superfluous.

In the case of a greater turbidity in the serum the titration should be given up, but this has very rarely occurred in the material here dealt with, despite the fact that titration has been made on several hundred specimens of sera.

In order to determine the accuracy of this titration with mercuric chloride I have in all titrations had the determinations made in duplicate, and for 300 cases these results have been plotted in a coordinate system as shown in Fig. 1.

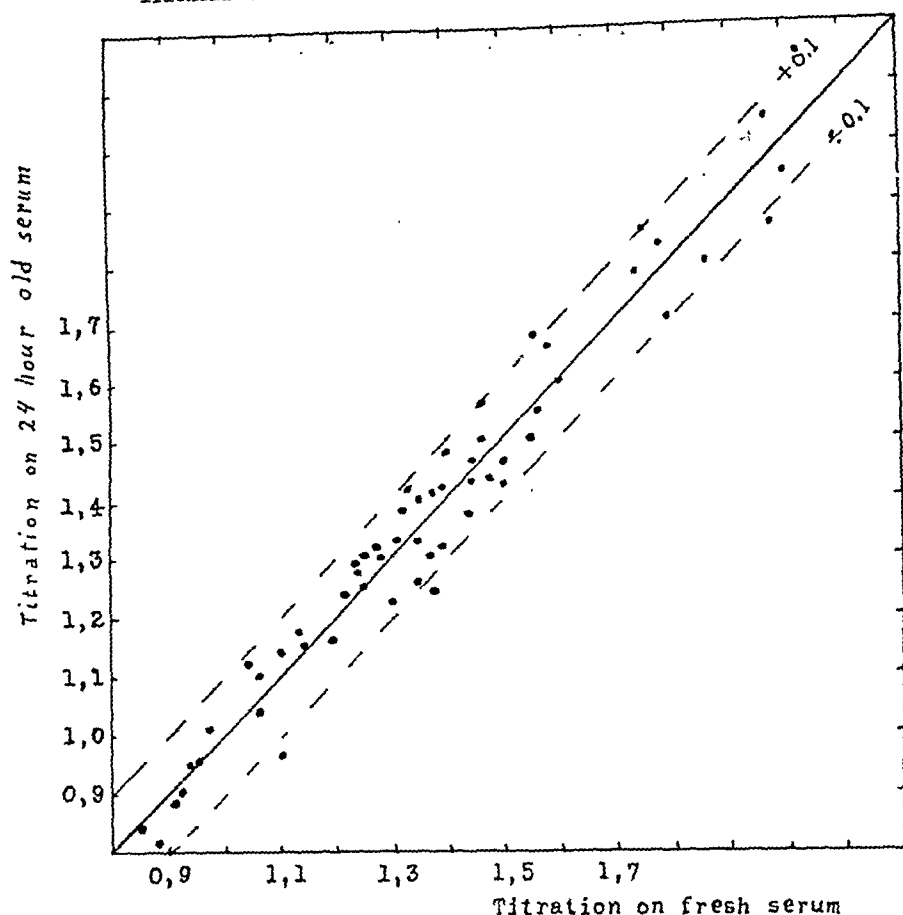


Fig. 3. Comparison between mercuric chloride titration on fresh and on 24 hour old serum (57 cases).

It will be seen that the titration deviations by the determinations in duplicate are only slight, and with the limitation ± 0.10 only a very small number of titrations will fall outside this area, in the case of the low positive values even as little as one, whereas in the case of the high negative values the dispersion is slightly greater.

In order not to let subjective conditions influence the results I have had two laboratory technicians titrate the same sera simultaneously and these results have been compared with my own titrations as shown in Fig. 2.

The deviations here do not seem to differ from the determinations in duplicate any essential degree.

Gros (4) and later Stolte (13) indicate that it is necessary to use fresh sera (Gros: not more than 12 hours old, and Stolte: not more

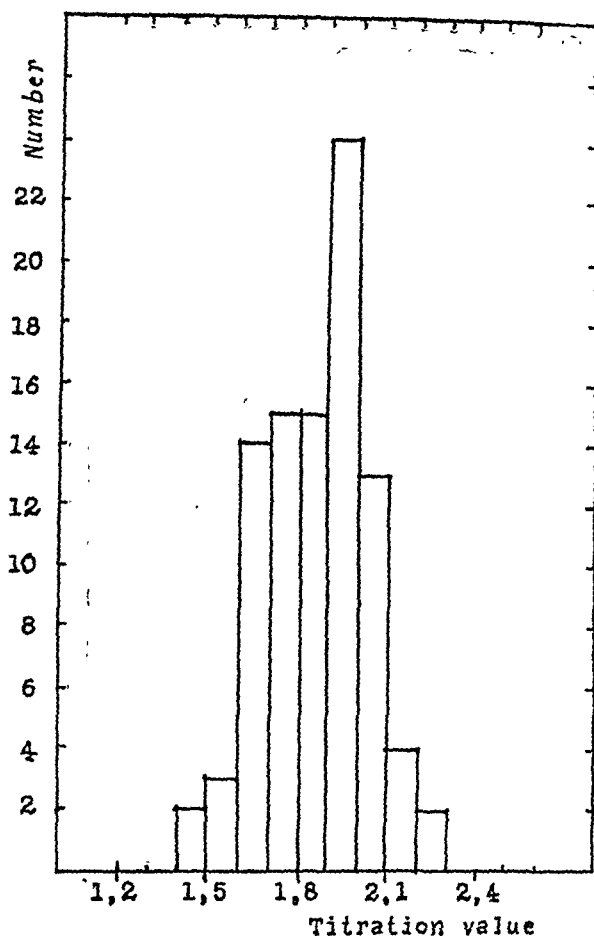


Fig. 4. Mercuric chloride values for 92 normal subjects.

than 3 hours old). I have therefore in 57 cases attempted titration of entirely fresh serum and compared the result with a titration made 24 hours later. The results are shown in Fig. 3, and again here there are no certain deviations from the values found at the determinations in duplicate. Thus I have not been able to confirm the investigations made by Gros and Stolte.

As normal values in this titration with mercuric chloride Stolte (13) has, on a material particulars of which are not given, given values from 1.6 upwards, and Hafström (7) indicates values from 1.2 to 2.0. By an examination on 92 sera from normal subjects I have found the conditions to be approximately the same as those found by Stolte. Fig. 4 shows the distribution and it will

be seen that the values fall between 1.4 and 2.3, or to be more exact, between 1.47 and 2.24, for which reason I consider it to be most appropriate with the accuracy found of the titrations to state the normal values as lying between 1.5 and 2.2. The values are distributed with the peak around 1.95 and with an average titration value of 1.85.

An examination of the normal values shows no difference between the two sexes, the average titration value for 41 normal women being 1.87 and the average value for 51 men 1.87. Nor does age seem to have any influence on the titration, the average titration values for the age groups being as follows: 10—20 years (15 persons) 1.85, 20—30 years (24 persons), 1.85, 30—40 years (18 persons) 1.84, 40—50 years (18 persons) 1.75, 50—60 years (8 persons) 1.84, 60—70 (9 persons) 1.89.

The mercuric-chloride titration and the Takata-Ara reaction are, as already mentioned, positive in various diseases, but it is above all in liver affections that they have been employed as an aid in the clinical work. In these lesions the tendency is towards pathological values in parenchymatous jaundice and normal values in obstructive jaundice. As has already been mentioned, the results vary greatly, probably depending on the highly different patient-materials employed in the investigations.

The patients with liver diseases are here divided into 4 groups: 1) the hepatitis group, which again is subdivided into 3 degrees, the mild cases of hepatitis with a duration of illness of less than 4 weeks, moderately severe cases of hepatitis with a duration of illness exceeding 4 weeks and, finally, the severe cases which show signs of passing into cirrhosis. 2) manifest cirrhosis of the liver irrespective of the cause. 3) cholelithiasis with icterus. 4) cancer of the pancreas with icterus.

All the cases have been tested regularly with mercuric-chloride titration and bilirubin in serum once a week and urobilin in urine twice a week (as will be seen from Figs. 6—8). For the determination of the bilirubin content in serum Jendrassik and Grof's method and for the determination of urobilin in urine Marcussen and Sv. Hansen's modification of Schlesinger's test have been employed; as + are designated positive reactions in the dilution 1 : 10 or upwards.

The outcome of the titration with mercuric hydrochloride in all these affections is presented in Table 1. The criterion for

Table I.

Account of titrations with mercuric chloride in lesions of the liver and bile ducts.

	Number of patients	Pathological titrations		Normal titrations	
		No.	%	No.	%
Hepatitis, acute	72	63	87.5 (91)	9	12.5
» slight	29	22	75.9 (83)	7	24.1
» moderate	19	18	94.7 (94)	1	5.3
» severe	24	23	95.8 (89)	1	4.2
Cirrhosis of the liver	14	14	100 (100)	0	0
Cholelithiasis	31	3	9.6 (11)	28	90.4
Cancer of the pancreas	5	0	0 (0)	5	100

normal and pathological titration values used in this tabulation is the lowest titration value found in the course of the illness.

From this tabulation it will be seen that of the hepatitis group comprising 72 cases the mercuric-chloride titration shows pathological values (*i. e.* < 1.5) in 87.5 % of the cases and that only 12.5 % present normal values (*i. e.* ≥ 1.5). Thus only 9 cases out of 72 show definite normal values and of these 7 are mild cases with a duration of illness of about 3 weeks, one case is of moderate severity and, finally, one is a severe case which terminated fatally and in which the postmortem examination demonstrated hepatitis subacuta, but in which the microscopic examination of the liver tissue presented a picture which deviates considerably from that usually met with in hepatitis and cirrhosis, and in which it is not possible to make a certain diagnosis by microscopic examination. However, the case is included in this group as the clinical course was typical of a malignant hepatitis.

This may give rise to a conjecture that it is possible to estimate the severity of the hepatitis directly from the titration value, and in order to decide this problem I have, in one figure, presented the graphs of the lowest titration values for each particular case in the various groups of hepatitis, as shown in Fig. 5.

From these graphs it is clearly seen that it is not possible to read the severity of the hepatitis directly on basis of the titration value, still, there is a clear tendency towards low titration values in the severe forms, so that a low titration value must arouse a certain suspicion of a more serious damage to the liver.

A really reliable basis for estimating the degree and course of

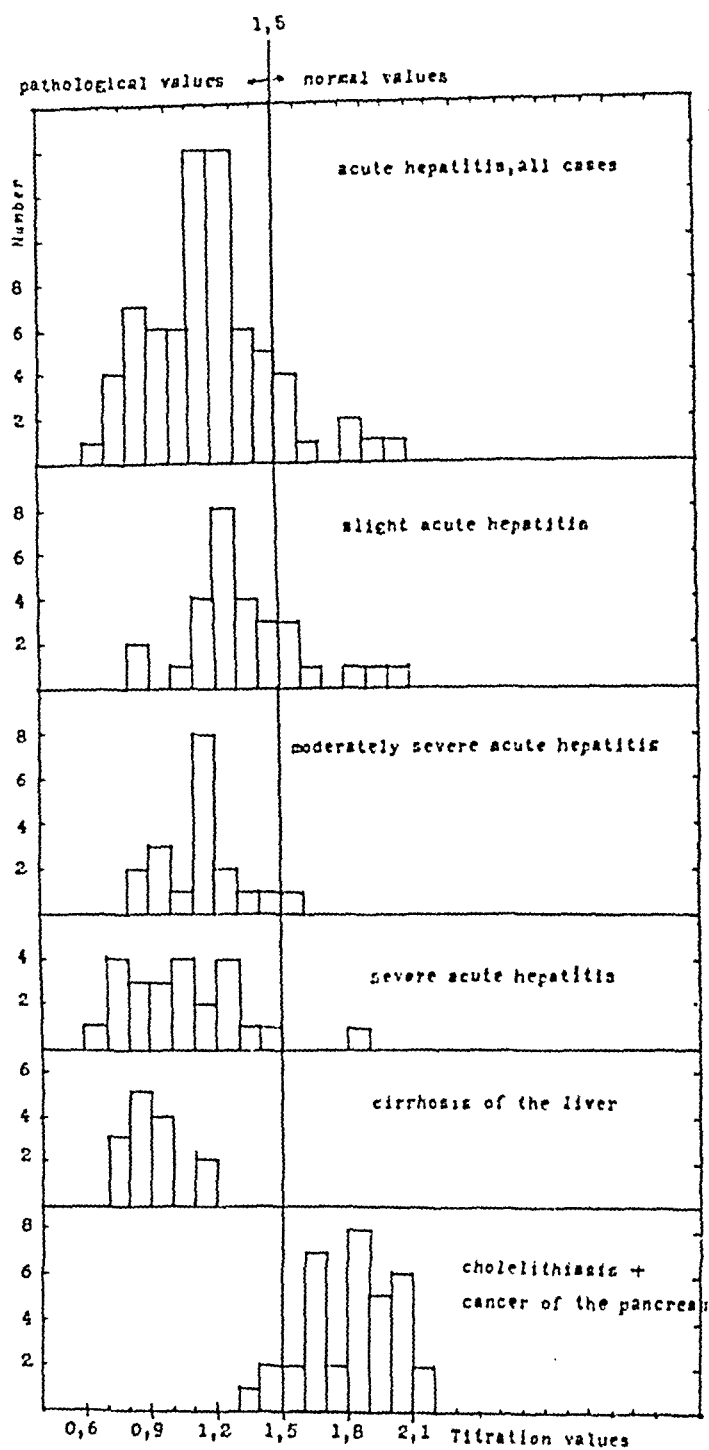


Fig. 5. Mercuric chloride titration of serum in different forms of liver and bile duct lesions.

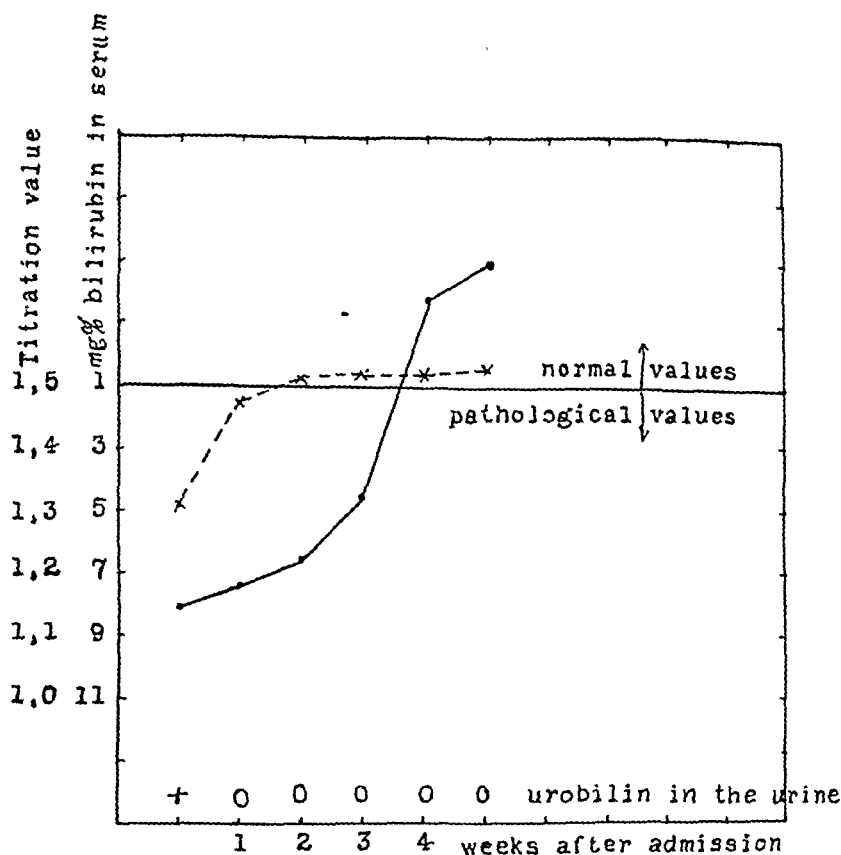


Fig. 6. The bilirubin value in serum and mercuric chloride titration of serum in a mild cases of acute hepatitis (case No. 353/46).

the hepatitis is only obtained by following several titration values during the course of the illness and observing the changes. In order to demonstrate this I have, in Figs. 6—8, plotted the curves from 4 typical cases which present the values of the mercuric-chloride titrations as compared with the bilirubin values in serum and the urobilin values in urine. Fig. 6 shows the position in the mild hepatitis in which the mercuric-chloride titration values as well as the bilirubin values in serum are progressing quickly and uniformly towards normal values. In Fig. 7 the position is seen to be the same as in the case of the moderately severe hepatitis, the progression being only somewhat slower. Fig. 8 shows the conditions in a severe case of hepatitis in which the bilirubin values rather quickly reached normal values, which induced the department to discharge the patient for convalescence despite

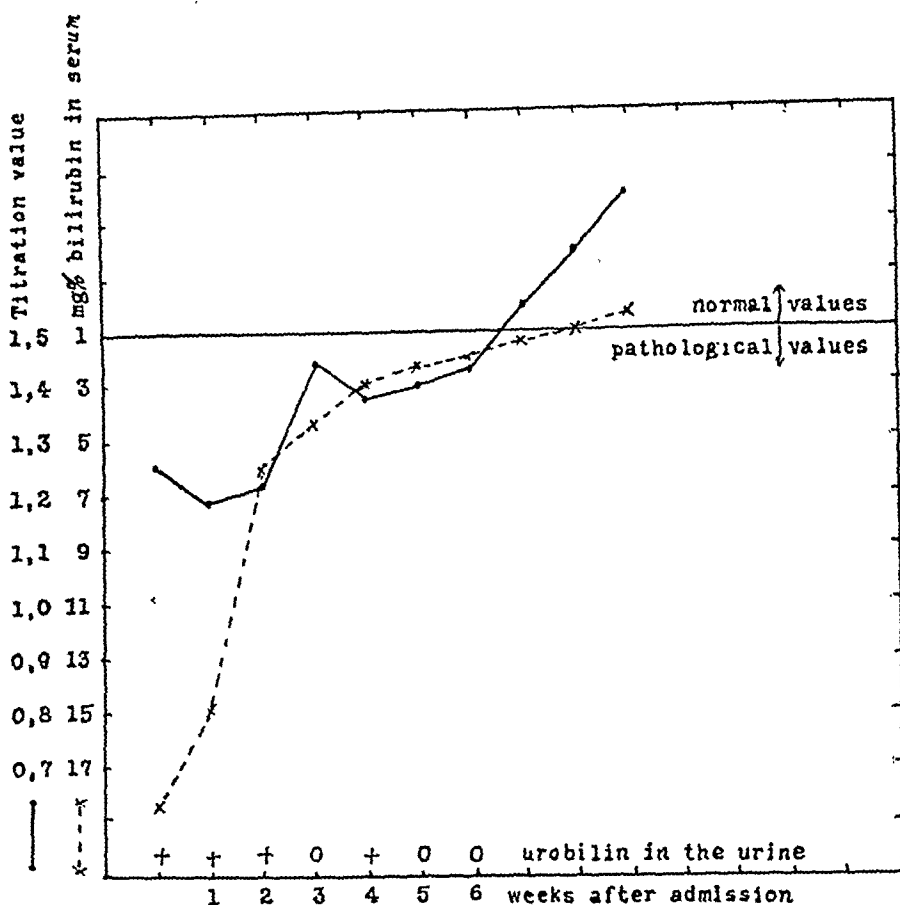


Fig. 7. The bilirubin value in serum and mercuric chloride titration of serum in a case of moderately severe acute hepatitis.

the fact that the titration with mercuric chloride showed values which remained at a rather low level, or rather, presented a slightly falling tendency. The patient was readmitted 2 months later in coma hepaticum and died, the titration value was now still lower and the bilirubin value high. The latter case demonstrates a feature which has made this ward keep these patients in bed until not only the bilirubin in the blood and the urobilin in the urine have presented normal values, but also till the titration with mercuric chloride has shown normal values, or at any rate constant values following a rising tendency. We believe that after having adopted this course we have observed a decline in the number of the very serious relapse cases which may occur in these diseases.

The next group of damage to the liver parenchyma is the

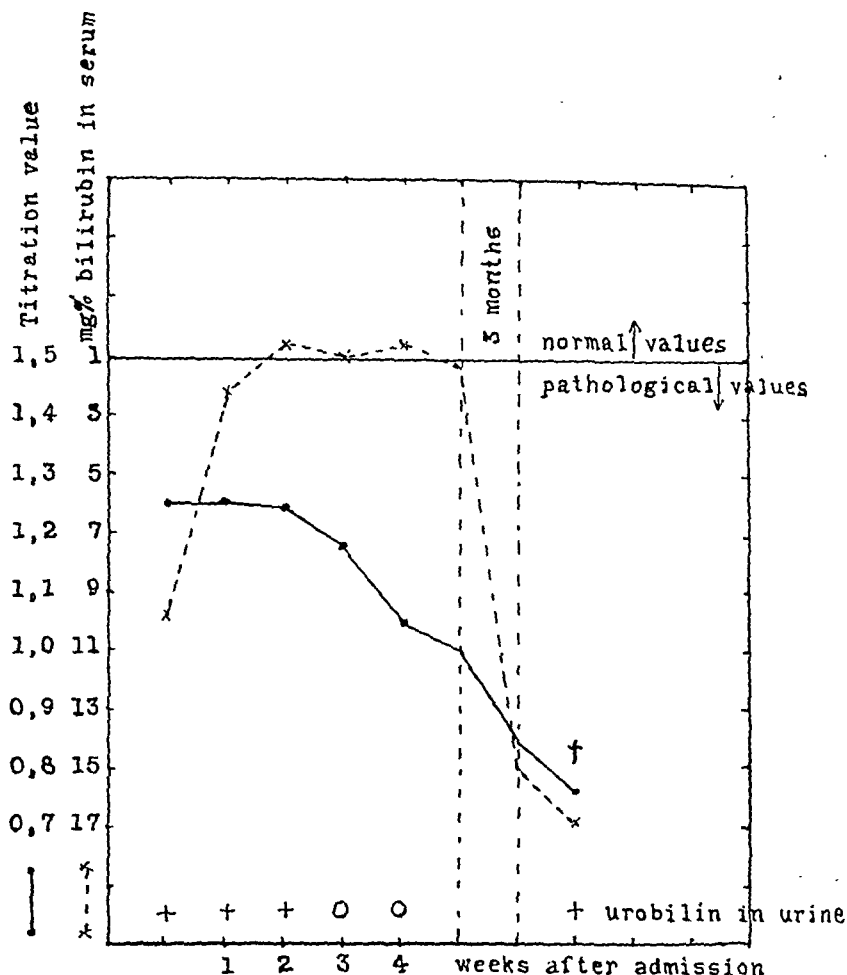


Fig. 8. Bilirubin in serum and mercuric chloride titration of serum in a case of severe acute hepatitis which terminated fatally.

cirrhosis group, which comprises all cases of cirrhosis of the liver irrespective of its origin (the cases mentioned in the hepatitis group, however, are not included here). These cases number altogether 14, which all of them presented pathological values with a considerable lowering of the precipitation limit, and thus all of them showed rather uniform, low titration values, as will be seen from Fig. 5.

Next the groups of icterus caused by stasis are to be dealt with in some detail, first the cholelithiasis group consisting of 31 cases of cholelithiasis with jaundice. 28 of these presented normal

titration values and only 3 presented definite pathological values. An examination of the latter cases demonstrated the precipitation limit is found to be only slightly lowered (1.30—1.40) and clinically the cases do not seem to differ from the other cases described in the group, more particularly, the cases have not had a specially protracted or violent course, nor have they been associated with fever of any great duration. The cases of cholelithiasis thus only presented definite pathological values in 9.6 % of the cases, while in 90.4 % of the cases the values were normal.

Finally, 5 cases have been included of cancer of the pancreas with gall stasis, which all of them presented normal values.

For the sake of surveyability the results have been tabulated in Table 1 and in Fig. 5 and from these it will be clearly seen that the titration of serum with mercuric chloride affords a valuable help in deciding whether a jaundice is due to a parenchymatous liver affection or a gall stasis, a low pathological titration indicating a liver affection and a normal value a gall stasis. If at the same time the simplicity of the method is stressed, its quick performance and simple way of recording the strength, these qualities should warrant its being increasingly used in clinical work.

Hafström (7) and Bjørneboe (1) have in contradistinction to Gros (5) and Vischer (15) indicated greater correctness with the Takata-Ara reaction than with titration with mercuric chloride. A comparison between the results obtained in this account with the mercuric-chloride titration and an account of the Takata-Ara reaction in liver lesions on a quite corresponding material (the figures in brackets in Table 1) seems to give the result that there is rather close agreement between the results. On basis hereof I have thus not been able to demonstrate any great difference in the sensitivity of the two reactions.

Diseases other than liver diseases are, as already mentioned, able to bring about changes in the serum proteins with a positive outcome of the mercuric-chloride titration; this is thus claimed to be the case in pulmonary tuberculosis, nephritis, lymphogranuloma inguinale, myeloma and sepsis. The material of these diseases has not been large enough to substantiate this, but titration with mercuric chloride of serum from persons arbitrarily chosen from among patients who have been admitted to the medical department has shown positive reactions to be of rather infrequent occurrence, and thus only sporadic positive values are in evidence in febrile

conditions. On the other hand it would seem that constant positive values are found in the acute rheumatic fever and in chronic polyarthritis. 20 cases of rheumatic fever in which blood was taken during the febrile stage showed an average titration value of 1.29 (1.20—1.59), and in chronic polyarthritis the average titration value for 12 cases was found, even apart from the febrile periods, to be 1.30 (1.00—1.57). In this connection it should just be observed that, as was expected, a constant relation has not been found to exist between the sedimentation rate of the blood and the titration with mercuric chloride.

In conclusion it is to be mentioned that Gros and Roussoulis (6) have attempted titration with Hayem's solution on ascitic fluid and there found low pathological values in patients with cirrhosis of the liver and normal values in ascitic fluid due to tumours, nephrosis or cardiac insufficiency. It would seem that only a small number of examinations would be required to confirm this, as titration with mercuric chloride of 5 cases of ascites due to cirrhosis gave an average titration value of 1.10 (0.9—1.2), whereas 2 cases of ascites due to a carcinoma presented titration values of 1.8 and 2.0.

Summary.

After a brief historical survey of the titration of serum with mercuric chloride, the method employed is described, *i. e.*, the titration given by Gros modified by Stolte: to $\frac{1}{2}$ ml serum and 1 ml 0.9 % NaCl 0.1 % HgCl_2 is added drop by drop till a permanent turbidity appears.

The normal values are found to be 1.5—2.2 ml; the accuracy ± 0.1 . In lesions of the liver and bile ducts mainly low pathological values are found in the parenchymatous liver lesions with jaundice, and mainly normal values in jaundice due to a mechanical obstruction of the bile ducts. Titration with mercuric chloride is therefore recommended as a reliable, speedy and easy method to aid in making the differential diagnosis between these conditions.

References.

1. Bjerneboe, M.: Acta Med. Scand. 1946: 124: 466. — 2. Grinsted, T.: Nord. Med. 1946: 31: 1879. — 3. Gros, W.: Med. Wochenschr. 1935: 82: 1151. — 4. Gros, W.: Klin. Wochenschr. 1939: 18: 781. —

Untersuchungen mit dem Tillmann'schen Verfahren herausstellen, der Ab- bzw. der Zunahme des Ascorbinsäuregehaltes im untersuchten Material zuzuschreiben sind.

Bei Beurteilung des Nikotinsäurehaushaltes musste berücksichtigt werden, dass von 100 mg nach Bandier's Vorschrift peroral eingeführter Nikotinsäure in 24 Stunden insgesamt 14 % ausgeschieden wird, wovon 70 % in den ersten 3 Stunden erscheint. Hingegen berichten Ellinger u. Shattock in ihrer bereits erwähnten Arbeit nach Belastung von 100 mg nur über eine Zunahme der Nikotinsäureausscheidung um 4—5 mg. binnen 24 Stunden, also in noch geringerem Verhältnis als in den Untersuchungen Bandiers. Da jedoch bei Untersuchung kleiner Mengen grosse Fehlermöglichkeiten nicht zu vermeiden sind, hatten wir parallel mit den Belastungsproben auch die Bestimmung der Nikotinsäure im 24-stundenharn ohne Belastung ausgeführt. Die Angaben im Schrifttum weichen auch hierüber ziemlich auseinander. So fanden Pearson u. Winegar, Harris u. Raymond, wie auch Swaminathan Tagesdosen von 5—6, Bandier solche von 2—3 mg. Alle Autoren wie auch Ellinger und Shattock stimmen darin überein, dass bei Nikotinsäuredefizienz die Ausscheidung täglich um 1 mg und noch darunter gefunden wird. Bei so widersprechenden Angaben des Schrifttums kann somit von einem normalen Nikotinsäuregehalte des Urins nicht gesprochen werden. Wir wandten zur Bestimmung die Anilin-Bromcyan-Methode von Pearson und Winegar mit dem Stufopparat von Pülfrieh an; da jedoch Bandier nachwies dass von den Nikotinsäurederivaten Nikotinsäureamid, Cozymase, Coenzym und besonders die Nikotinursäure nur nach Hydrolyse mit starker Lauge für die Bestimmung erfassbar werden, haben wir Pearson und Winegar's Verfahren hiermit ergänzt.

Bei Vergleich der Ergebnisse nach Belastung mit Vitamin B₁ stellt sich heraus, dass die an unserer Klinik übliche Diät allein nach der Methode von Magyar keine B₁-Hypovitaminose verursacht, ebensowenig entsteht Mangel nach längerer Behandlung mit Sulfonamiden. Bei Penicillinkuren haben wir die Belastung nach Einverleibung von 5—5.5 Millionen O. E. ausgeführt, nur ein Patient erhielt bei der Untersuchung 11 Millionen Einheiten. Wie wir anlässlich des Vitamins B₂ bereits erwähnten, ist infolge des verminderten Wertes der derzeitigen Ernährungsverhältnisse ein geringer Mangel an B₁ auch bei gesunden Menschen zu finden, so müssen die am Anfang der Penicillindosierung gefundenen Aus-

Aus der II. Medizinischen Klinik der Universität, Budapest. Direktor:
Prof. Dr. E. Haynal.

Störungen im Vitaminhaushalt bei längerer Penicillinbehandlung.

Von

Dr. LADISLAUS MOSONYI und Dr. ELISABETH OBLATT.

(Bei der Redaktion am 8. September 1947 eingegangen.)

Im Laufe des letzten Jahres konnten wir bei zwei unter Penicillinbehandlung stehenden Kranken solche allgemeine Symptome feststellen, welche mit ihrer Krankheit keineswegs unmittelbar in Zusammenhang gebracht werden konnten und welche meistens den Erscheinungen einer beginnenden B₁-Hypovitaminose ähnelten. Im ersten Fall erschienen in der dritten Woche der Behandlung einer an Endocarditis lenta leidenden Patientin — als sie bei einer täglichen Dosierung von 500,000 O. E. im fraglichen Zeitpunkt die Gesamtmenge von 11 Millionen O. E. bereits überschritten hat — Extremitätsschmerzen von ziehendem Charakter, Parästhesien, allgemeine Muskelschwäche, Appetitlosigkeit und patellare Reflexdifferenz, solche Zeichen also, die von Robinson am 10.—14. Tage einer experimentellen B₁-Hypovitaminose beobachtet werden konnten. Die Patientin war seit Beginn der Penicillinbehandlung stets fieberfrei, irgendwelche Decompensationssymptome liessen sich nicht feststellen, Appetit, Ernährungszustand blieb bis dahin unverändert und das oben beschriebene Bild war mit etwa anatomischen Veränderungen nicht zu erklären. Durch die perorale Darreichung von täglich 3×3 mg Vitamin B₁ wurde die Verminderung ihrer Beschwerden und das Verschwinden der Reflexdifferenz erzielt. Wir sahen uns deshalb veranlasst die von Magyar beschriebene Vitamin B₁-Belastungsprobe durchzuführen, durch welche dann eine ausgesprochene B₁-Hypovitaminose entdeckt werden konnte.

Nach Vorschriften des Verfassers werden in vier nacheinanderfolgenden zweistündlichen Zeitabständen je 2 mg Vitamin B₁ den Versuchspersonen beigebracht und im entsprechenden Urin die ausgeschiedene Vitaminmenge bestimmt. Nach Magyar sollte unter normalen Verhältnissen 20—25 % der einverleibten Vitaminmenge in jeder Periode ausgeschieden werden; die Ausscheidung bleibt während des ganzen Versuches konstant bzw. zeigt eine unregelmässige Vermehrung oder Verringerung. Im Fall einer bestehenden Hypovitaminose steigt von einem niedrigen Ausgangspunkte die Ausscheidung stufenweise empor, weil die früher einverleibte Menge auf vitaminärmere Gewebe stösst. Im unseren erwähnten Versuch war die Ausscheidung 7, 9, 15 bzw. 19 %; die Hypovitaminose wurde somit einwandfrei feststellbar.

Ein zweiter Patient (subphrenischer Abszess) erhielt 7,000,000 O. E., als er über ähnliche Beschwerden klagte. Ausser den obigen Erscheinungen konnte bei ihm auch eine hartnäckige Tachykardie beobachtet werden. Durch 16 mg Vitamin B₁, auf vier Tage verteilt, gelang es auch seine Beschwerden zu beseitigen.

Ellinger und Shattock behandelten eine leichte Pharyngitis bei einer Patientin, die früher mehrmals pellagroide Symptome aufwies, täglich mit 7,000 O. E. Penicillin per os; am 10. Tage der Behandlung traten an der Zunge Zeichen auf, die der Mangelkrankheit der Hunde »Black-tongue« ähnelten und welche Erscheinungen sich tatsächlich nach Darreichung von 200 mg Nikotinsäure vollkommen zurückentwickelten. Nach einigen Tagen konnten sie bei der Patientin mit versuchsweise per os gegebenen Penicillin dieselben Symptome auslösen, wogegen das parenteral zugeführte Penicillin in dieser Hinsicht keine Wirkung hatte. Der Zustand, welcher übrigens mit einer mit Belastungsprobe feststellbaren Nikotinsäuredefizienz Hand in Hand einherging, wurden von Ellinger und Shattock als Folge der Veränderung der Mund- und Darmflora aufgefasst, ähnlicherweise wie es nach Einnahme grösserer Mengen von Succinyl-Sulfathiazol und Sulfoguanidin in mehreren Fällen ebenfalls von Ellinger beobachtet wurde.

Wegen des immer breiteren Gebietes der Penicillinbehandlung, anderseits wegen der sich stets verlängernden Kuren und grösseren Dosen sahen wir uns veranlasst systematisch zu untersuchen ob die obigen von uns selbst beobachteten und die von Ellinger und Shattock beschriebenen Fälle als zufällige Koinzidenzen oder

aber als zwangsmässige Begleiterscheinungen jeder Penicillinbehandlung aufzufassen sind? — Aus technischen Gründen befassten wir uns zuerst mit Fragen bezüglich der wasserlöslichen Vitamine; Versuche mit Vitamin K sind im Gange. Zuerst mussten wir uns darüber klar werden, ob das Penicillin etwa eine unmittelbare Wirkung auf diese Vitamine nicht ausübt? Es wurden Versuche angeordnet, wonach 20 O. E. Penicillin (nach Fleming erreicht sogar das intravenös eingespritzte Penicillin nur in der ersten halben Stunde ein Serumkonzentration von 4 Einheiten pro ml) und bestimmte Mengen Vitamin B₁, B₂, C und Nikotinsäure mit gleichem Teil (1 ml) physiol. Kochsalzlösung bzw. Blutserum im 37° Brutschrank eingestellt wurden. Nach 3, 2, bzw. einer Stunde zurück erhielten wir die gesamte angewandte Menge, mit Ausnahme der Versuche mit Vitamin C, wo dies sich nach 3 Stunden um etwa 18 %, nach zwei Stunden um 12 % verminderte, jedenfalls ganz parallel in beiden Lösungen, mit oder ohne Penicillin. Diese Versuche beweisen, dass das Penicillin sogar in stärkeren Konzentrationen keine direkte Wirkung auf die wasserlöslichen Vitamine ausübt. Durch die Anwesenheit von Blutserum wird daran nichts geändert. Diese Versuche wurden mit gleichem Ergebnis viermal wiederholt. — Es musste ebenfalls festgestellt werden, ob die an unserer Klinik während der Wintermonate übliche Diät an und für sich nicht unzureichend sei, wodurch Hypovitaminosen in Erscheinung treten könnten. Endlich mussten auch diese längst bekannten Tatsachen mit in Kauf genommen werden, nämlich dass jene Krankheiten, die neulichst besonders mit Penicillin behandelt werden, mit den Hypovitaminosen sogar zwei Berührungsstellen besitzen: teils kann die Ansteckung selbst sehr oft nur im schon von vornherein vitaminarmen Organismus zur Geltung kommen — das bezieht sich besonders auf das Vitamin C —, andererseits bedeutet der Verlauf der Krankheit, besonders die Fieberzustände, einen gesteigerten Vitaminbedarf. Die Kontrollversuche wurden deshalb folgendermassen angeordnet: wir untersuchten den Zustand des Vitaminhaushaltes bei Kranken nach wenigstens einer Woche klinischen Aufenthaltes (Gruppe A); im Hinblick auf die Angaben von Ellinger und Shattock wählten wir als Gruppe B jene, die mit Sulfonamiden behandelt wurden; endlich führten wir Untersuchungen auf solchen Personen durch, die vor Penicillinbehandlung standen (Gruppe C); diese Untersuchungen wurden meistens noch im fiebernden Zustande vorgenommen. Um eine etwaige

vitaminvermindernde Wirkung des Fiebers auszuschalten, setzten wir die Versuche nur bei solchen Personen fort, die sich nach Beginn der Penicillinbehandlung sofort entfieberten. Die während und nach der Penicillinbehandlung erhaltenen Angaben sind in Gruppe D zusammenfasst.

Zur Feststellung der Hypovitaminose wählten wir Belastungsproben; nach Ishihara verlässt etwa 50 % der eingespritzten Vitamine während 24 Stunden den gesättigten Organismus. Der grösste Teil dieser Menge wird in den ersten drei Stunden ausgeschieden. Diese in kurzer Zeit ausführbaren Methoden wiedergeben also das zuverlässigste Bild über den Sättigungsgrad der Körpergewebe. Die B₁-Belastungsprobe wurde nach dem erwähnten Verfahren von Magyar durchgeführt. Zur Vitamin B₂-Belastung haben wir die Methode von Góth verwendet: Die Versuchsperson erhält in nüchternem Zustand 11 mg B₂ intravenös. (Beflavin Roche); nachher wird die im Urin in zwei Stunden ausgeschiedene Menge des Vitamins B₂ vor Analysenquarzlampe mit einer Standardlösung titriert. Diese Menge beträgt nach Góth unter normalen Sättigungsverhältnissen ebenfalls ungefähr 20—25 % der ursprünglich einverleibten. Wie aus folgenden Tabellen ersichtlich, beobachteten wir in gesunden Personen nur eine Ausscheidung von 18—20 %. Diese Verminderung kann durch die Ernährungsstörungen erklärt werden, die in der Nachkriegszeit allgemein zur Geltung kommen, sodass nur die Ausscheidung unter 15 % als pathologisch aufgefasst werden kann.

Die Hypovitaminose-C wird im Urin durch die bekannte Dichlorphenol-indophenol-Reagensmethode von Tillmanns, andererseits mit der Belastungsprobe von Góth untersucht. Diese letztere beruht auf folgendem Prinzip. 300 mg Ascorbinsäure werden intravenös eingespritzt und der C-Vitamingehalt des Serums vor und zwei Stunden nach der Injektion bestimmt. Bei Individuen ohne Vitamin C-Defizit nimmt der normale 0.7—1.2 mg % betragende Blutserumgehalt nach 300 mg i.v. gegebener Ascorbinsäure mindestens um 0.5 mg % zu (Góth). Zur Vermeidung etwaigen unspezifischen Reduktion waren wir mit Rücksicht auf Dobszay's Erfahrungen, wonach die vorhandene Ascorbinsäure in den fraglichen Lösungen durch Vorbehandlung mit starker Lauge zerstört wird. Die übrigbleibende, nicht von Vitamin C stammende Reduktion ist somit von der Ascorbinsäure zu trennen. Auf diese Weise überzeugten wir uns, dass die Veränderungen, die sich im Serum und Urin im Laufe unserer

gangswerte von ungefähr 15 % als normal angesehen werden, umso mehr da hierbei die auf Vitaminmangel so charakteristischen, regelmässig aufsteigenden Ausscheidungskurven fehlen.

Tabelle 1.

B₁-Belastungsproben nach Magyar.

Vitamin B₁-Ausscheidung im Urin. Zweistündlich 2 mg eingespritzt, Urin zweistündlich gesammelt; in % der einverleibten Menge

Gruppe	Fall				
A	1	14	21	20	20
	2	19	24	26	25
	3	23	22	24	24
	4	19	20	23	21
	5	20	25	27	25
	6	18	22	19	20
B	7	19.5	21	18	24
	1	25	27	29	28
	2	23	23	26	29
C	3	27	31	30	31
	1	16	16	17	19
	2	15	18	14	18
	3	17	15	20	16
D	4	16	19	15	18
	1	7	9	15	19
	2	6	11	13	25
	3	9	10	13	21
	4	5	9	11	16
	5	8	13	13	20
	6	10	14	16	21

Tabelle 2.

Vitamin C-Belastungsversuche im Serum nach Góth.

Gruppe	Zahl der Fälle	Nüchterner Serum- C-Wert in mg %	2 Stunden nach 300 mg C i.v. in mg %
		durchschnittlich	durchschnittlich
A	5	1.25	2.15
B	4	0.81	2.3
C	9	1.58	2.72
D	8	1.15	1.38

Auf 300 mg Belastung zeigt sich in den Gruppen A, B und C im Vitamin C-Werte der Sera eine normale Schwankung. Bei solchen, die mit Sulfonamiden behandelt wurden, ist der niedrige (nach Góth's Angaben zwar zwischen normalen Grenzen bleibende), wie auch bei bevorstehender Penicillinbehandlung der hohe Ausgangswert auffallend. Diese Erscheinungen sind wahrscheinlich der geringen Zahl unserer Fälle zuzuschreiben. Demgegenüber ist bei solchen, die durchschnittlich eine Penicillinkur von 5,000,000 O. E. durchgemacht haben, die Vitamin C-Avidität

der Gewebe sehr ausgesprochen, worauf der normalen Zunahme von 0.6 mg % gegenüber eine solche hinweist, die mehr um die Hälfte geringer ist. Entschiedene und hochgradige Abweichungen werden auch anlässlich der Bestimmungen der Reduktionsfähigkeit des Urins gegen Dichlorphenol-indophenol beobachtet. Wohl ist die Reduktionsfähigkeit der Harne von Kranken nach Behandlung mit Sulfonamiden gewissermassen auch verlängert, doch bleibt diese Wirkung weit unter dem Reduktionszeitdurchschnitt jener Urine, die von penicillinbehandelten Kranken stammten. Aus Fig. 1 ist ersichtlich, dass zwischen der Verringerung des Ascorbinsäuregehaltes im Urin und der verbrauchten Penicillinmenge eine so gut wie vollkommener Parallelismus besteht, wobei weniger die Behandlungsdauer, als die einverleibte Penicillinmenge die Hauptrolle zu spielen scheint.

Die Verzögerung der Harnreduktionszeit beginnt durchschnittlich um den Zeitpunkt, als in der Behandlung die erste Million O. E. erreicht wird.

Tabelle 3.

Reduktionszeit im Urin gegen Dichlorphenol-indophenol.

Gruppe	Zahl der Fälle	Durchschnittliche Reduktionszeit
A	7	2'
B	4	3.5'
C	12	2.5'
D	13	13'

War die Reduktionszeit des Urins um mehr als 10', verlängert, erzielten wir mit 120 cg Vitamin C in vier Tagen, ohne Unterbrechung der Penicillinbehandlung die Rückkehr zum Normalen. Danach blieb die Reduktionszeit des Harnes unverändert, wenn wir täglich zugleich 30 cg Vitamin C zuführten. Wenn wir aber die Dosierung versuchsweise aussetzten, kam die Defizienz in 6—7 Tagen wieder in Erscheinung.

Tabelle 4.

Vitamin B₂-Belastungsproben nach Góth.

Gruppe	Zahl der Fälle	Vitaminausscheidung im Harn nach Zufuhr von 11 mg i.v.
		durchschnittlich in mg %
A	5	16
B	3	17.5
C	9	19.5
D	11	9.9

Die auf Vitamin B₂ bezüglichen Untersuchungen erwiesen, dass während der Penicillinbehandlung nach Belastung mit 11 mg Riboflavin die Ausscheidung sich auf die Hälfte des Normalen verringerte. In den Gruppen A, B und C sind keine Unterschiede auffindbar.

Die Untersuchung der Nikotinsäureausscheidung im 24-stündigen Harn zeigt, dass diese Angaben nicht zu verwerten sind.

Tabelle 5.

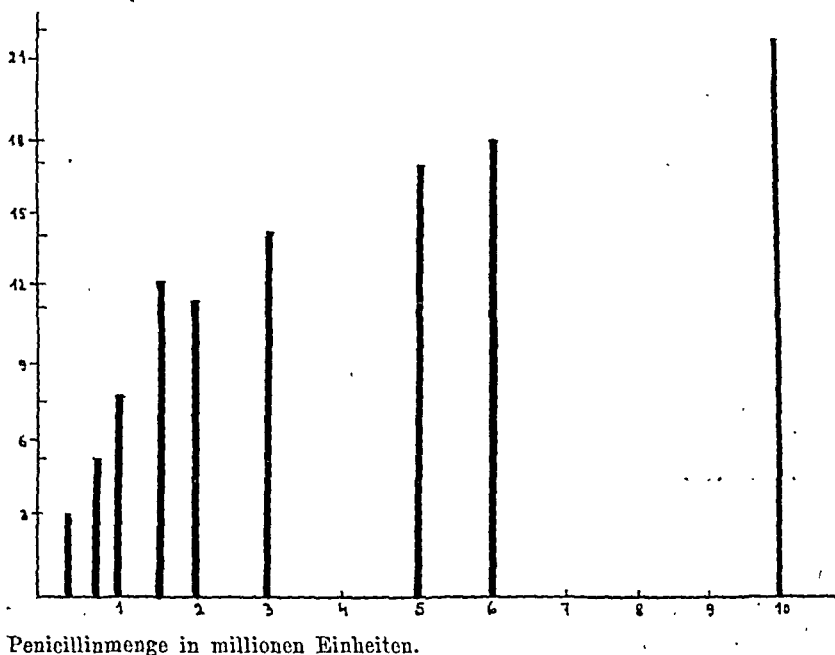
Untersuchungen bezüglich auf das Nikotinsäurehaushalt.

Gruppe	Zahl der Fälle	24-Stundenausscheidung im Urin in mg %	Nach Belastung mit 100 mg Nikotinsäure in mg %
A	5	2.8	11.4
B	5	4.21	14
C	—	—	—
D	6	3.3	6.09

Einerseits scheinen die im Schrifttum als normal angegebenen Werte noch nicht von allgemeiner Geltung zu sein, hauptsächlich infolge der Verschiedenheit der Ernährungsverhältnisse, andererseits sind die Werte, die wir bei unseren Untersuchungen in den A, B und D Gruppen erhielten, kaum voneinander verschieden. Hingegen weisen die Belastungsproben mit 100 mg Nikotinsäure per os bei mit Penicillin behandelten Patienten eine bedeutende Verminderung der Ausscheidung auf, so dass wir in diesem Sinne ebenfalls von einer Steigerung des Vitaminbedarfes der Gewebe reden können. Im Falle von Ellinger und Shattock war die Ausscheidung noch mehr verringert, doch zeigten sich bei ihrer Patientin deutliche pellagroide Zeichen, während wir solche nie beobachten konnten. Wir müssen uns darauf beschränken unsere Versuchsergebnisse zu wiedergeben, ohne dieselben für Nikotinsäuredefizienz bezeichnend erklären zu können.

Danach führten wir in vitro und gleicherweise in vivo Untersuchungen darüber durch, ob den Vitaminen in der bakteriziden Wirkung des Penicillins im Serum eine Rolle zukommt? Nach Knight zeigen Staphylococcusarten, welche auf synthetischen Nährboden schlecht gedeihen, auf Einwirkung von Nikotinsäure und Vitamin B₁ ein vorzügliches Wachstum. Mueller machte bezüglich des Bac. diphtheriae dieselbe Erfahrung. Auf das Wachstum des Proteus (Fildes) und der Shigella paradyseuteriae Sonne (Fraser, Topping und Sebrell) ist Nikotinsäure dermassen wirk-

Fig. 1. Zusammenhang zwischen der Dichlorphenol-Indophenol-Reduktionszeit des Harnes und der einverleibten Penicillinmenge im Durchschnitt von 21 Fällen. Minuten



sam, dass dadurch das Vitamin biologisch nachzuweisen und sogar quantitativ zu bestimmen ist."

Durch Zusatz in vitro von je 1 mg B₁, B₂, C und Nikotinsäure zum penicillinhaltigen Blutserum änderte sich die Auslöschzone an der Heatley-Agarplatte nur durch Zufügung von Vitamin C; es muss aber betont werden, dass sogar mit reiner Vitamin C-Lösung eine Auslöschzone entstand, deren Durchmesser Hand in Hand mit der Konzentration des Vitamins sich änderte, war aber deutlich kleiner, als die Zone hervorgerufen durch eine Lösung, die neben gleicher Menge Vitamin C auch Penicillin enthielt. Stepp und Kühnau äussern sich zwar für die wichtige Rolle des Vitamins C in der Überwindung allerlei Infektionen, doch findet man im Schrifttum keine Angaben die für eine direkte bakterizide Wirkung sprechen würden. Wir wiederholten unsere Versuche fünfmal mit demselben Resultate, trotzdem halten wir es für möglich, dass es sich in unserem Fall um eine besondere Empfindlichkeit des in unserem Besitz befindlichen Staphylococcus.Oxford-Stammes handelt, eben deshalb fühlen wir uns zur Stellungnahme weder über die bakterizide Wirkung der Ascorbinsäure, noch im Hin-

blick des Synergismus von Vitamin C und Penicillin nicht berechtigt. Bei gleichzeitiger parenteraler Zufuhr von Vitaminen und Penicillin konnte keine Steigerung in der bakteriziden Wirkung sogar bei Anwendung stärkerer Konzentrationen wahrgenommen werden.

Endlich behandelten wir gesunde Ratten ebenfalls mit Penicillin. Die Tagesdosis des subcutan eingespritzten Penicillins betrug 2,800 O. E. pro Kg-Körpergewicht. Wegen der kleinen Zahl unserer Versuchstiere (3) kann man keine endgültigen Folgerungen ziehen, trotzdem stellte es sich eindeutig heraus, dass die Vitamin B₂-Ausscheidung — die am Anfang des Versuches und in den Kontrolltieren durchaus 70 % der einverleibten Menge (1.5 mg) betrug, nach einer Woche Penicillinbehandlung auf 5 % herabfiel. Mit Vitamin B₁ und Nikotinsäure erhielten wir keine eindeutigen Resultate, dagegen fiel auf, dass der Ascorbinsäuregehalt im Urin auf ein Drittel des der Kontrolltiere sank.

Summary.

Clinical evidences led the authors to assume deficiencies in some vitamins during prolonged penicillin treatment. This is particularly striking in the case of vitamin C. The deficiency could be detected about on the end of the first week of usual penicillin treatment (160,000 O. U. daily). Similar alterations could be found in the household of other water-soluble vitamins. The investigations were carried out using the test dose-methods of Magyar (B₁), Góth (B₂ and C) and Pearson and Winegar (nicotinic acid). The deficiencies can be explained: 1. By changes in the mouth and intestinal flora. 2. The cysteine of the prosthetic group of the penicillin producing glutathion can play a rôle in the cellular respiration as cocarboxylase = B₁, flavenzyme = B₂, cozymase = nicotinic acid and redox-system = vitamin C do it; hencefore can the increased amount of glutathion disturb the equilibre of these systems. The 30—35 % of ingested penicillin, which is not demonstrable in the urine may play a rôle in that process. 3. Penicillin may directly influence cellular respiration and can produce thus biochemical changes in the organism.

Literatur.

Bandier: On Nicotinic Acid. Copenhagen. Einar Munksgaard. 1940. — Crandon, Lund und Dill: JAMA. 115. 1637. 1940. — Dobszay: Kl. Wschr. 21. 522. 1942. — Ellinger und Shattock: Brit. Med. Journ. 611. 1946. — Fleming: Penicillin. Butterworth and Co. London. 1946. — Goth: Zschr. f. Vit.forsch. 10. 15. 1940. — Góth: Schweiz. Med. Wschr. 74. 1246. 1944. — Ishihara: JAMA. 117. 1396. 1947. — Magyar: Orv. Lapja: 1946. 406. — Pearson und Winegar: Zschr. f. Vit.forsch. 10. 238. 1940. — Robinson: JAMA. 114. 439. 1940. — Knight, Fildes, Fraser, Topping, Sebrell, Swaminathan zit. nach Bandier.

Note on the Pathogenesis of Jaundice.

By

TORBEN K. WITH.

Copenhagen.

(Submitted for publication March 25, 1948.)

In this Journal Volume 128, pp. 25—41 I published a paper in which the following theory of the pathogenesis of jaundice was advanced: If the pressure in the bile passages rises above a certain limit the liver cells cannot overcome this pressure and cease to secrete against it into the bile canaliculi, and instead they secrete by means of a secondary secretory mechanism into the lymph spaces of the liver. The same secondary secretory mechanism was proposed to be the cause of icterus neonatorum.

I have recently read a paper of J. C. McCarrel, S. Thayer & C. K. Drinker (*Am. J. Physiol.* 1941, 133, 79—81) which shows that the auxiliary hypothesis of a secretory mechanism from the blood plasma into the liver lymph is quite unnecessary. These authors have clearly shown that the protein content of the liver lymph is equal to that of the blood serum, and that the parenchyma cells of the liver virtually are bathed in blood plasma. As bilirubin is firmly bound to the albumin fraction of the plasma proteins, the primary action of the liver cells in the bilirubin part of the bile formation unquestionably is to break up this linkage to the serum albumins. But in the case of lymphogenous jaundice they are not able to do this — from one cause or the other — and the bilirubin passes into the liver lymph together with the plasma proteins being still bound to the albumin fraction.

Thus the rôle of the lymph in the pathogenesis of jaundice is not to be regarded as a sequel of a special secondary secretoric mechanism of the liver cells but simply as a consequence of the fact that the liver cells are bathed in blood plasma.

Simultaneously I should like to mention the paper of H. E. Thompson & B. L. Wyatt (*Arch. Int. Med.* 1938, 61, 480—495) which I did not know at the time my above-mentioned paper appeared. In this interesting paper it is shown that jaundice can be induced in man by daily intravenous injections of great doses of bilirubin (10—15 mg. per kg. and day). Serum bilirubin concentration up to 12.5 mg. per 100 ml. in the interval between the injections and pronounced jaundice of the skin and mucous membranes may be induced in this way. Unfortunately these authors did not perform more detailed biochemical studies as their primary object was to study the influence of induced jaundice on chronic polyarthritis.

Correction to:

Pancreatic Disease Combined with Vitamin-K-Refractory Hypoprothrombinemia. (1947: 129; 33.)

By

HOLGER BEGTRUP.

(Submitted for publication February 9, 1948.)

The patient reported on died a short time ago (from hematemesis). Post-mortem examination revealed a normal pancreas, but a rare malformation of her liver (Spiegel's lobe) which had at the operation presented itself as a pancreatic growth. Microscopical examination of liver tissue and spleen showed Bantis' disease — the cirrhotic affection of the liver being the probable cause of the hypoprothrombinemia.

It was the incorrect diagnosis of a tumor of the pancreas, which inspired the investigations by H. Begtrup and E. Tage-Hansen (Acta Physiologica Scand. 14: 189—194; 1947), and in which it was demonstrated that pancreatectomy in dogs caused a K-vitamin-refractory hypoprothrombinemia.

From the Pediatric Clinic of Karolinska Institutet,
Norrtulls Hospital, Stockholm.
(Chief: Professor A. Wallgren.)

The Accuracy of Some Clinical Methods (Autenrieth, Stufen and Sicca) of Determining Hemoglobin Values — a Comparison.

By

PETTER KARLBERG.

(Submitted for publication August 26, 1947.)

This investigation concerns the more satisfactory clinical methods practised in Sweden for the determination of hemoglobin. Its objects are:

1. To determine the errors relevant in routine work and how they can be avoided, or at least diminished.
2. To calculate the accuracy of the various methods and to determine which conclusions we are justified in drawing, under varying conditions, from a difference between two hemoglobin values.
3. To attempt to decide if any of these methods is superior as regards accuracy and practicability.

The following hemoglobinometers were used in this investigation: Autenrieth-Königsberg's colorimeter, Pulfrich's Stufenphotometer and the Sicca hemometer.

Autenrieth was taken as representative both of Zeiss-Ikon and Autenrieth, both having the same working principles and very similar manner, since the latter is considered to be somewhat more accurate (Enghoff 1937, Bierring 1940, Ehrenberg 1943).

The Sahli method, which is still used in some places, was not included, since it was demonstrated in earlier investigations (Sundberg 1938, Grotepas 1941, Østerskov Jensen 1943, Sørensen 1943, amongst others) to have an error in the method up to $\pm 10\%$, and is therefore con-

sidered to be definitely inferior to the other methods. It has even been classed by a number of writers with Tallqvist's method.

We find in the literature that several investigations have been made to assess the accuracy of the Autenrieth, Zeiss-Ikon and Sicca, as well as the Stufen methods. They were shown to have approximately the same accuracy, with a deviation of between $\pm 1\%$ and $\pm 5\%$ (Hesse 1937, 1938, Heilmeyer-Mutius 1938, Sundberg 1938, Humperdinck 1939, Sorensen 1941, Grotepas 1941, amongst others). When the Sicca method has been investigated, it has always been preferred, since it lacks the so-called hematin error and is, moreover, practical. No investigation has, however, been made in which each working stage of various methods has been consistently examined and carried out, and a comparison made of the accuracy under varying conditions on the basis of statistically calculated errors. Moreover, the Stufen-method has not been investigated as a practical routine method, although this appears, nevertheless, to be justifiable since it is relatively simple. Furthermore, according to Zeiss' Swedish representative, the Stufen photometer is to be found in the majority of the large hospitals in Sweden (50 in Stockholm and 70 in the country).

A. Examination of the Different Working Stages With Respect to the Possible Analytical Errors as Well as to the Working Time.

I. The Following Working Stages Are Discussed.

- 1) Preparation for taking the test.
- 2) Taking of the test.
- 3) Reading time (interval between the taking of the test and the reading).
- 4) Work with the hemometer and errors in reading.
- 5) Subsequent work.

1. Preparation.

Autenrieth: Filling of the test tubes with 1.980 (1.990) mm³ dilution fluid, 1/10 Normal HCl., for mixing. Time: 25 secs.

Errors: The automatic pipettes used for filling the test tubes are liable to errors up to 1 per cent if, for example, the liquid is blown out of the pipette instead of being allowed to run out.

Stufen: = Autenrieth, but 0.04 % ammonia — or 0.1 % soda solution is used as a dilution fluid. Time: 25 secs.

Sicca: No preparations. Time: 0 secs.

2. Taking of the test.

Autenrieth: 20 mm³ of capillary blood are sucked up in a pipette, introduced into the HCl. solution in the test tube for mixing, and shaken. The blood is hemolysed and acid hematin is formed. Time: 31 secs.

Errors: In order to reduce the price, the capillary pipettes are usually sold without a guarantee for a certain maximal error, and the gradation can therefore be subject to not insignificant errors. Control weighing with quicksilver of 30 pipettes from the laboratory of Norrtull's Hospital, purchased during the year from various firms, showed the following distribution within the margins of error:

Margins of error	0—1 %	1—2 %	2—3 %	3—5 %
No. of pipettes	19	7	3	1

It should, however, be noted that a broken point alters the volume of the pipette.

Even a satisfactorily graduated pipette can give a value up to approximately 5 % too low, if it is not dried before use. This is an obvious fact, but owing to the lack of pipettes, it often occurs. If it is not possible, for practical reasons, to dry the pipettes before each test, errors can be avoided by first sucking up blood to the limit, blowing out the blood and then taking the blood test in the usual way. (The pipette is thus "washed" with the patient's blood.) It is unnecessary to point out that insufficient mixing, with resultant coagulation, gives false values.

Stufen: = Autenrieth, with the difference that oxyhemoglobin is obtained instead of acid hematin. The dilution given by Heilmeyer, 25/2,475 has been altered to 20/1,990 in order to make use of the laboratory equipment in use in Sweden. *Time:* 32 secs.

Sicca: Approximately 30—40 mm³ of capillary blood are sucked up with a special hand pipette. A steel rod is moistened with the blood in the pipette, dipped in a powder reagent (consisting of sodium oxalate, saponin and sodium hyposulphite). An approximate amount of the reagent adheres to the rod and is inserted once more into the pipette, where the reagent is dissolved in the blood. If the result is not read within the following few minutes, the pipette is placed in a special reservoir tube. The blood is stabilized and hemolysed, and reduced hemoglobin is formed. *Time:* 48 secs.

Errors: If too much reagent is added, the blood becomes cloudy and viscous and air-bubbles are then easily formed in the fluid layer when the wedge chamber is filled (see below). Reading is thus made more difficult or impossible. After investigations with increasing quantities, the suitable quantity was found to lie between 1 and 10 mg., thus between quite wide limits. This corresponds to a moderate powdering, without lumps, of the steel rod. It is practically impossible to take too small a quantity.

Insufficient mixing causes the blood to become granular (a not uncommon fault with beginners).

If the reagent is old, or has been stored for a time uncorked, it becomes unusable (the hyposulphite is oxidised by the air).

These errors can, however, easily be avoided, when the worker has become familiar with the method.

A stage common to all three methods, which can give rise to errors, is the actual *technique of drawing the blood*. This was, however, in-

vestigated recently by Sørensen (1941), who studied very carefully the effects of the technique. His work is therefore referred to. Since the importance of the pressure on the finger-tip in taking a test is often a topical question in assessing the accuracy of an Hb. value, Sørensen is quoted (Fig. 1) showing that moderate pressure has very little effect.

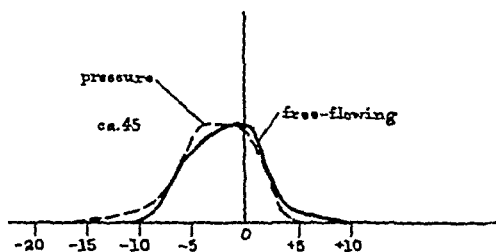


Fig. 12. Influence of pressure on the finger (Blood taken approximately 0.3–10 mins. after incision). The pressure and free-flowing blood tests are, essentially, equally satisfactory.

Fig. 1. Taken from G. Sørensen, Nord. Med. 1941: 10: 1118.

3. Reading time (interval between taking the test and reading).

Autenrieth: Since the acid haematin darkens afterwards, and it is this colour strength which is measured, the reading must take place at a fixed time in relation to the taking of the test. As a rule, an interval of 5 or 60 minutes is used. The hemometer is corrected for the particular time according to a standard. Time is gained by reading after 5 minutes, but there is a loss of accuracy. Since the darkening takes place more rapidly in the beginning (see Fig. 2), a fixed difference in time gives a greater error with a value taken after 5 minutes than after 60 minutes. In hospital routine, where the delay is used for other work, the 60 minutes' value is used as a rule. This is the case in the present investigation.

The process of darkening is also dependent on the temperature (Barkan-Olesk 1937, Enghoff 1937, Heilmeyer-Mutius 1938). According to Enghoff, each degree of variation in temperature gives a variation of 0.5 % in the Hb. value in ordinary conditions of investigation.

Time: not noted.

Stufen: Time for reading: according to choice.

Sicca: According to the literature (Hesse 1937, 1938, Sundberg 1938, 1945, Ehrenberg 1943, Kaada 1945) the reading can be taken at a time independent of the taking of the test, according to the last-mentioned, up to 6–8 hours later.

Since, however, even a very slight dehydration of the undiluted blood should give a noticeable rise in the Hb. value, the effect of the reading time was studied.

On a series of persons (31) 6 tests were taken on each, and in each 6-test series, 3 were immediately, and 3 after 1–2 hours, during which time the blood pipettes were kept in special tubes in the way prescribed.

The difference between the average for the first three and the last three values was calculated. The average difference (\bar{d}) with its average error ($\epsilon\bar{d}$) was found to be $+1.71 \pm 0.51$ in percentage of the Hb. value obtained¹, a statistically established increase of the tests read

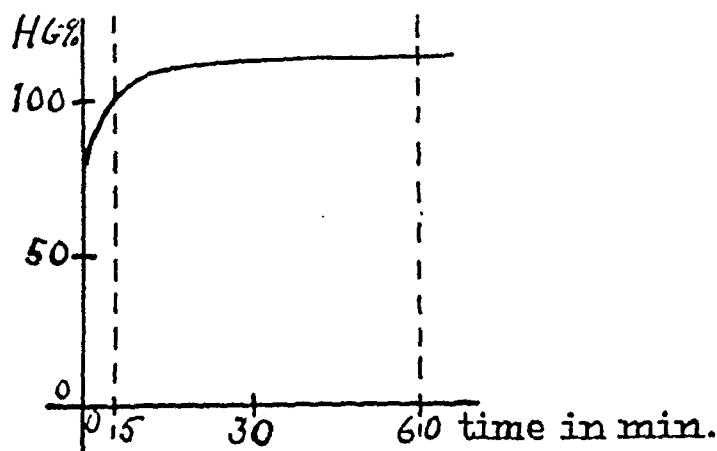


Fig. 2. An after-darkening curve for acid hematin.

after 1—2 hours (greater than 3 times its average error). The difference is, however, small but sufficiently large not to allow the test to stand unnecessarily before reading.

¹ The following statistical formulae and signs are used:

Double determinations

d = difference between two values

n = number of differences

$S(d)$ = total of differences

\bar{d} = average difference = $\frac{S(d)}{n}$

σd = standard deviation of differences = $\frac{\sqrt{S(d-\bar{d})^2}}{n-1} = \sqrt{\frac{S(d)^2 - \frac{[S(d)]^2}{n}}{n-1}}$

$\epsilon\sigma d$ = average error in standard deviation of differences = $\frac{\sigma d}{\sqrt{2n}}$

$\epsilon\bar{d}$ = average error in average difference = $\frac{\sigma d}{\sqrt{n}}$

If the average difference, \bar{d} , is less than 3 times its average error, $\epsilon\bar{d}$, ($\bar{d} = 0$) becomes

σ_x = standard deviation in the individual determination, the error in the individual determination = $\frac{1}{\sqrt{2}} \times \sigma d$

$\epsilon\sigma_x$ = average error in the error of the individual determination = $\frac{1}{\sqrt{2}} \times \epsilon\sigma d$

Average error in the average number for 3 determinations = $\frac{1}{\sqrt{3}} \times \sigma_x = \frac{1}{\sqrt{6}} \sigma d$

Summation of 2 errors σ_x and σ_y , becomes $\sigma_{x+y} = \sqrt{(\sigma_x)^2 + (\sigma_y)^2}$

The extrapolation calculation is according to Bonnier-Tedin.

4. *Work with the hemometer and errors in reading.*

Autenrieth: The hematin solution is transferred from the reservoir mixing tube to a chamber, which is colorimetrically compared with a fluid wedge, and readings taken. The percentage of hemoglobin is calculated with the aid of a correction curve for a certain standard and reading time. The chamber is then washed. *Time:* 55 secs.

Errors in reading: see page 105.

Stufen: The oxyhemoglobin solution in the mixing tube is reduced by the addition of a "pinch" of hyposulphite. It is transferred to a 0.5 cm. chamber and is read in the Stufen photometer from the colour similarity in the filter S 57 (corresponding to a wavelength of 572 μ). According to Heilmeyer, the extinction obtained is recalculated to hemoglobin percentage for a certain standard according to the formula:

$c = E_{d}^{S57} = 1.0 \text{ cm} \times 15.8 \text{ g. Hb. in 100 ml. blood.}$ In order to express the hemoglobin level in percentage of Haldane's standard (100 % = 13.8 g. % Hb.) the extinction is multiplied by $\frac{15.8 \times 100}{0.5 \times 13.8} = 2.29 \times$

100. Since the gelatine filters are not constant, this method, as well as the others, should be standardized. *Time:* 77 secs.

Errors: The amount of hyposulphite, "a pinch", varies. By reading with increasing values of hyposulphite, the upper limit was found to lie at 10 mg. Larger quantities give turbidity in the solution and increase the light absorption (extinction). Reduction occurs even with the addition of 1 mg. and there is, therefore, practically no risk of using too small a quantity. The suitable amount is soon discovered.

The time elapsing between the addition of the hyposulphite and the reading is limited, since the hemoglobin is gradually oxidised by the air. If, as in this investigation, the smaller quantity of fluid is used, 2,010 mm³ instead of 2,500 according to Heilmeyer, the chamber is not entirely filled, although sufficiently above the level of the beam of light through the chamber. Oxidation should reach the level through which the beam of light passes. In a series of readings at varying times, and after addition of varying quantities of reagent, this oxidation did not, however, become apparent during the first twenty minutes, even if only 1—2 mg. hyposulphite were added. 10—20 mixing tubes can thus simultaneously be supplied with hyposulphite and the readings can take place successively.

Errors in reading: see page 105.

Sicca: The blood is transferred from the pipette to a wedge-shaped glass chamber, which is formed between a glass plate and a "glass wedge". Reading takes place by adjusting the liquid wedge until a similarity in colour has occurred against a glass standard, illuminated by an electric bulb built into the hemometer. The hemoglobin value is read directly on a scale, expressed in per cent of Haldane's standard (100 % = 18.5 vols. % δ O₂ = 13.8 g. % Hb.). The "glass wedge" and the glass plate are then cleaned extremely carefully. *Time:* 105 secs.

Errors: Coarse turbidity as above (page 101). If the glass plate or the "glass wedge" are not completely dried, lighter areas appear in the fluid wedge. Even the slightest contamination of the "feet" of the "glass wedge" gives increased values.

Errors in reading: see below.

Errors in Reading.

Since all three methods are based on direct colorimetry, it is inevitable that, on reading, a certain spreading must occur around the true value. In order to assess the degree of spread, and whether any difference occurs in the difficulty of reading in the three methods, double readings were made.

From the differences, the error in one reading and the average error in three readings (as a rule, 3 readings were made per test) were made for each of the three methods.

Table I.

Reading error in the Autenrieth, Stufen and Sicca methods with internal differences.

All values given in % of Hb. values obtained.

Method	No. of differences (n)	Average difference between 2 readings ($\bar{d} \pm \epsilon \bar{d}$)	Average error in 3 readings $\left(\frac{1}{\sqrt{6}} \times \sigma_d \pm \frac{1}{\sqrt{6}} \times \epsilon \sigma_d \right)$	Difference in reading error between 2 methods
	1	2	3	4
Autenrieth (A)	126	0.07 % \pm 0.118	\pm 0.53 % \pm 0.034	A-St -0.11 % \pm 0.052
Stufen (St)	126	0.25 % \pm 0.139	\pm 0.64 % \pm 0.040	St-Si +0.14 % \pm 0.014
Sicca (Si)	390	0.17 % \pm 0.061	\pm 0.50 % \pm 0.018	A-Si +0.03 % \pm 0.038

From the values obtained (see Table I) it is evident that the average error for three readings is approximately $\pm 0.5 - 0.6$ % of the hemoglobin value read. Consequently, no difference of practical significance exists between the three methods.

It is perhaps necessary to point out at this stage that the bilirubin content in the blood gives too high values on the hemoglobin content by Autenrieth's method. According to Enghoff (1937) there is an increase of 7 % in colour index 120. Using Sicca's method, this can be reduced by a built-in gold filter (Hesse 1938). Nor is it so evident with Stufen's method, since a certain wavelength is used at which the bilirubin has a very low absorption.

5. Subsequent work.

Autenrieth: Cleaning and drying of the pipette and mixing tube.

Time: 57 secs.

Stufen: = Autenrieth.

Time: 57 secs.

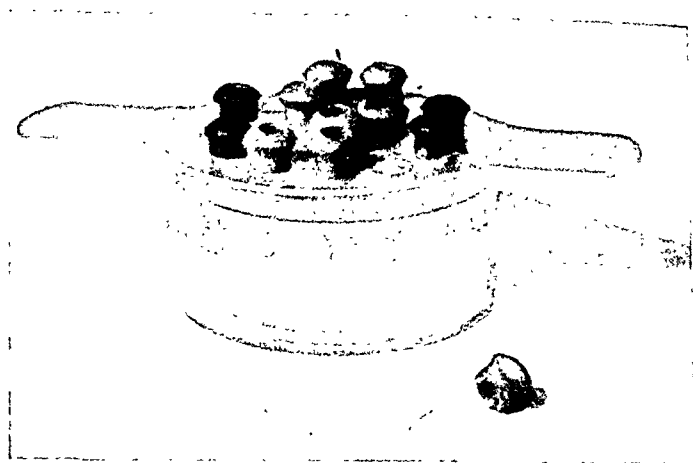


Fig. 3. Cleaning container for Sicca pipettes.

Sicca: Cleaning of the pipette, which is easier than in the case of Autenrieth's capillary pipette. Time: 25 secs.

This time can be considerably reduced if a metal plate with holes is used (see Fig. 3). This is placed over a bowl with weak alkali solution, and each pipette is placed in a hole, after the glass chamber has been filled. All the pipette can then be cleaned simultaneously in this position, by sluicing under a tap, dipping in a bowl with alcohol (or even ether), and drying in a thermostat.

II. Time-Studies.

Time-studies are shown in Table II. Since these aim at illustrating the routine work in a hospital, the interval between the taking of the test and the reading was not included, since this time is used for other work.

The total time is lowest for Autenrieth and somewhat longer for Sicca and Stufen. But these differences are small and irrelevant.

If, however, the time is divided into that of the laboratory nurse and that of the laboratory technician, the circumstances differ (see Table II). The Sicca method takes longest for the nurse — approximately 1 minute longer than the Autenrieth and Stufen methods, i. e. nearly double, since the nurse herself in series of examinations must, between each reading, carefully clean Sicca's glass plate and "glass wedge". We can therefore understand that the Sicca method has not been met with any great enthusiasm on the part of many nurses.

If, however, the five-minute reading time in the hematin methods is used in order to obtain a rapid result — and the delay must then, at any rate partially, be included in the working time — the advantage of Sicca's method, with immediate reading, is clearly shown. This was demonstrated in 1938 by Sundberg, in whose time-studies the delay was included.

Table II.

Time-studies in work with Autenrieth, Sicca and Stufen methods.

Stage of work	Autenrieth			Stufen			Sicca		
	No. of time determinations	Average time in seconds		No. of time determinations	Average time in seconds		No. of time determinations	Average time in seconds	
		Nurse	Technician		N.	T.		N.	T.
1 Preparation	120	—	25	120	—	25	—	—	—
2 Taking of test	106	31	—	106	32	—	106	48	—
3 Reading time	—	—	—	—	—	—	—	—	—
4 Work with hemometer.	111	55	—	101	77	—	120	105	—
5 Subsequent work	155	—	57	155	—	57	108	—	25
Work done by N.	—	86	—	—	109	—	—	153	—
Work done by T.	—	—	82	—	—	82	—	—	25
Total working time:	168			191			178		

B. Errors in Individual Determination.**1. Errors in the Method.**

After examination of the different stages in the three methods, it is evident that, even if care is exercised, it is necessary to allow for certain errors in the hemoglobin values obtained. In order to obtain a measure of the accuracy of the methods, their errors = the error in each separate determination, were calculated by means of double determinations. Such errors found for the three methods (see Table III, col. 3) lie between $\pm 1.85 - \pm 2.36$ % of the hemoglobin value obtained. As regards the Sicca method, the error is statistically probably greater than for the Autenrieth method (difference $> 2 \times$ average error). The Stufen method, however, does not differ statistically from the other two.

The error in the Autenrieth test (and in other hemoglobin tests with acid hematin) should not be greater than that obtained by means of the double determinations, when the so-called hematin error is not evident. Barkan (1937) and others have thus demonstrated that the darkening of the hematin can have a variable course in individuals with the same hemoglobin level, up to a variation of ± 5.3 %.

Table III.

Error in method = error in each individual determination with Autenrieth, Stufen and Sicca methods, with internal difference.

All values given in % of Hb. values obtained.

Method	No. of differences (n)	Average diff. between double determinations ($\bar{d} \pm \epsilon_d$)	Error in method $\left(\frac{1}{\sqrt{2}} \times \sigma_d \pm \frac{1}{\sqrt{2}} \times \epsilon_{od} \right)$	Difference in error in method between 2 methods
	1	2	3	4
Autenrieth (A)	42	0.10 % \pm 0.402	\pm 1.85 % \pm 0.202	A-St -0.16 % \pm 0.300
Stufen (St)	41	0.63 % \pm 0.717	\pm 2.01 % \pm 0.222	St-Si -0.35 % \pm 0.265
Sicca (Si)	130	0.38 % \pm 0.277	\pm 2.36 % \pm 0.146	A-Si -0.51 % \pm 0.249

2. Different Methods.

In order to assess the agreement between the three methods and thus to ascertain the influence of the hematin error on routine work, the hemoglobin level was determined in 124 persons, with simultaneous Autenrieth, Stufen and Sicca tests. The three series thus obtained with hemoglobin levels can only be compared two by two. Three combinations are thus possible: Sicca/Autenrieth, Autenrieth/Stufen and Sicca/Stufen. In each of the three combinations, each pair of corresponding hemoglobin values was drawn on a system of coordinates, where the abscissa was graduated for one method and the ordinate for the other. Each pair of values was thus represented by a dot (see Fig. 4).

Since the methods are worked out in such a way that they should give the same value for the same hemoglobin level, these dots should lie on a straight line. Since, however, each value can have a certain error (error of method), these dots will not all lie on the line, but on either side. The position of this line has been fixed for each combination by regression calculation (according to Bonnier-Tedin) and drawn on the diagram.

The standard deviation obtained for the three combinations can be seen in Table IV, col. 2, and it is found (col. 3) that no statistical difference exists between them.

Since, as stated above, the error for the Autenrieth method is found to be statistically probably lower than for the Sicca, it could have been expected that the diffusion about the regression line would be less for Autenrieth/Stufen than for Sicca/Stufen. That no such difference is found could be dependent on the fact that the error for the Autenrieth method can, in reality, be greater than that obtained through the double determinations. This increase in the error for the

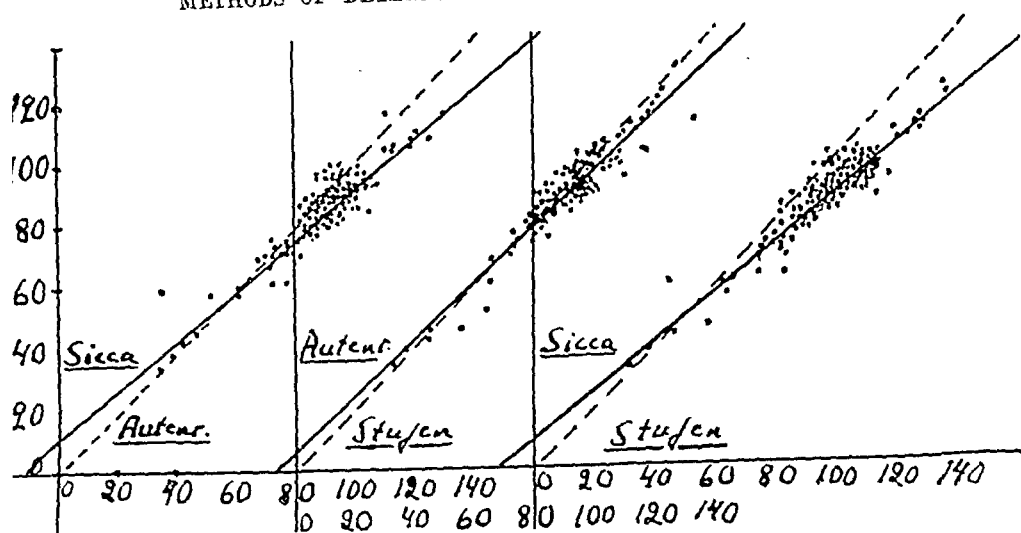


Fig. 4. Graphical comparison between the Hb. values obtained with 1) Sicca/Autenrieth, 2) Autenrieth/Stufen and 3) Sicca/Stufen. Determinations made on 124 persons.

Table IV.

Spreading between Hb. values determined by Autenrieth, Stufen and Sicca methods.

All values given in % of Hb. value obtained.

Combination of 2 methods	No. of determinations per method (n)	Standard deviation about the extrapolation line $[\sigma(y-Y) \pm \epsilon_{\sigma(y-Y)}]$	Difference between spreading in 2 combinations
	1	2	3
Auth./Stufen (A/St)	124	5.86 % \pm 0.372	A/St—Si/A —0.82 % \pm 0.563
Sicca/Auth. (Si/A)	124	6.68 % \pm 0.423	Si/A—Si/St —0.40 % \pm 0.581
Sicca/Stufen (Si/St)	124	6.28 % \pm 0.398	A/St—Si/St —0.42 % \pm 0.515

Autenrieth method can be explained by the fact that the so-called hematin error has appeared. With the maximal limits of ± 5.3 % (Barkan 1937), the hematin error raised the error of the Autenrieth method to approximately the same degree as for the Sicca method. Thus the errors for the three methods are approximately the same, i. e. ± 2.3 %.

Since each of the three methods, as shown above, has an error of approximately ± 2.3 %, the diffusion between the methods (= the diffusion about the extrapolation line) should, from a statistical viewpoint, be approximately ± 3.3 %. The investigation gave, however, a value of approximately ± 6.2 %. This can, however, be explained, partly by the fact that the errors of method are calculated on normal material, but the diffusion between the methods is calculated on collected normal and pathological material, and also by the fact that a straight line agreement between the methods can scarcely be expected.

3. *Standardization.*

If, in a series of determinations of the hemoglobin values, two different hemometers are used for each person, and the values obtained are inserted into a coordinate system with a similar scale on the abscissa and the ordinate — as in Fig. 4 — and the extrapolation line is calculated, this line should have a slope of 45° for exactly the same standardization of the hemometers (both methods give the same value).

If, however, one method gives higher values at a high hemoglobin level, and lower values at a low hemoglobin level, the extrapolation line will be influenced in relation to the line of 45° .

In Fig. 4, the three extrapolation lines form an angle with the 45° line, which thus means a standardization which is not in agreement. In the investigation, however, the Autenrieth colorimeter used was recently re-standardized according to the usual custom, and the Sica hemometer was newly purchased. The Stufen photometer was not specially standardized, but the extinction coefficient, as indicated by Heilmeyer, was directly used.

It is not surprising that an exact standardization is not obtained, if it is considered how a standardization of a hemometer, for routine use, is usually performed. A series of blood tests is read off in the hemometer in question, and on the basis of the values thus obtained, a correction curve is drawn. Since each reading can, as has previously been pointed out, contain a certain error, and each series seldom consists of more than 8—10 blood tests, such a standardization will entail an error.

In order to assess the size of this standardization error in, for example, the Autenrieth colorimeter, 6 standardization series, which were kindly placed at our disposal by the chemical laboratory of Karolinska Sjukhuset, were analysed. Each series consisted of 6—10 pairs of readings, on which an regression calculation, as described above, had been carried out. Since the regression line is the correction curve sought, the spreading of the pairs of values about the line of extrapolation expresses the error in standardization. The errors thus obtained in the 6 standardization series are given in Table V. The average error was ± 2.75 % of the hemoglobin value. The correction curve is usually, however, drawn freehand, and the standardization error should, therefore, be somewhat greater, approximately ± 3.5 % of the hemoglobin value.

Table V.

Standardization errors in 6 standardizations of the Autenrieth colorimeter.

All values in % of the Hb. values obtained

Standardization	No. of readings per hemometer	Spreading about extrapolation line standardization errors
1	2	3
1	10	± 2.66 %
2	9	± 2.72 %
3	10	± 1.56 %
4	7	± 5.60 %
5	9	± 1.97 %
6	6	± 1.97 %

Average error ± 2.75 %

4. Different Observers.

The subjective interpretation of the similarity in colour also contributes to the uncertainty of a hemoglobin value. According to Hesse (1938) a variation of approximately ± 3 % is obtained with different observers. Approximately the same figures were found in the present investigation in tests involving a few small series.

The probability that a difference found between two hemoglobin values is caused by an actual difference in the Hb. levels is dependent upon the conditions under which the two values were obtained. On the basis of the order of magnitude of errors calculated previously in an individual determination, the error in the difference between two Hb.

Table VI.

Limits for the possibility that a difference found between 2 Hb. values, under different conditions, exists.

All values given in % of Hb. value obtained.

S = same D = different

Observer	Standardization (Same Standard)	Hemometer	Method	True difference exists with a probability of	
				67 % if diff. >	95 % if diff. >
S	S	S	S	3.3 %	6.6 %
S	D	D	S	4.5 %	9.0 %
S	S	D	S	5.5 %	11.0 %
S	S	D	D	6.2 %	12.4 %
S	D	D	D	6.2 %	12.4 %
S	D	D	D	7.0 %	14.0 %
S	D	D	D	7.6 %	15.2 %

values was calculated for different conditions of examination. If the difference is once or twice greater than the calculated error in the difference, an actual difference in the Hb. value exists with at least 67 and 95 % of probability respectively. The probable limits under varying conditions are seen in Table VI.

If greater accuracy than that expected is desired, it can be obtained by carrying out a double test. The error will then be $\frac{1}{\sqrt{2}} = 0.707$ times lower.

In comparing two Hb. values, obtained in different hospitals, it is necessary to remember that *different standards* are used. Thus, for example, four different standards exist in the Stockholm hospitals: Haldane's 100 % = 13.8 g. % hemoglobin; Eng-hoff's 100 % = 15.3 g. %; the German standard 100 = 16.0 g. %; and one where 100 % = some value between 15 and 16 g. %. This means that, in two different hospitals, 75 % and 87 % indicate the same hemoglobin value.

C. Choice of Method.

For Hospital Use.

Autenrieth and *Sicca* (hematin error included in *Autenrieth*) are comparable in accuracy, if the hemoglobin determination is carried out by competent laboratory technicians and if, in the *Autenrieth* method, the one-hour reading time is used, as well as pipettes controlled by weighing. Under such conditions, the advantages of the *Sicca* method against the *Autenrieth* are fairly insignificant, working thus with an approximate quantity, undiluted blood, an approximate amount of reagent and an indefinite reading time. Nevertheless, the disadvantage of longer working time for the nurse remains. There is, therefore, no direct reason, under such conditions, to replace *Autenrieth* by *Sicca*.

The reservation should perhaps be made that the bilirubin error and reading error dependent on the yellow colouring of the lens of the eye in elderly persons is reduced with the *Sicca* method (Hesse 1937, 1938). If it is desirable to avoid these errors and the hematin error, but the *Sicca* method is unacceptable owing to its practical disadvantages, *Stufen* can be used as a fully serviceable routine method, possibly giving somewhat more accurate values than the two other methods. Since the *Stufen* method is very similar to that of *Autenrieth*, a change-over is relatively simple.

A certain familiarity with and experience of the photometer is, however, necessary.

If, however, the determinations are carried out by less experienced personnel, the accuracy of the Autenrieth method is decreased more than that of Sicca, since the technique of drawing the blood in the Sicca method less easily gives rise to unobserved errors. Incorrectly graduated pipettes or reading after five minutes also markedly decreases the accuracy of the Autenrieth method. Under such conditions, the Sicca method is to be preferred.

The statements made above concerning the Autenrieth method are also valid for Zeiss-Ikon, although the accuracy of the latter is probably somewhat less, partly on account of the use of varying sources of light and the greater difficulty in reading.

For the doctor in private practice, the Sicca method is the best of those examined. Its advantages are increased in that no longer working time is required, since all the stages are usually performed by the same person. The importance of a fresh Sicca reagent must, however, be emphasised.

However, a small disadvantage of the Sicca method must be pointed out, namely that almost double the amount of blood is required than with the Autenrieth method. This has a certain importance, at least where children are concerned.

The *Sahlbi* method which, as a result of earlier investigations, has shown to possess considerable inaccuracy, as well as the impossibility of making several readings from the same test, must be considered inferior to the other methods.

Summary.

Three methods for the determination of the hemoglobin values: Autenrieth (diluted acid hematin); Stufen (diluted reduced hemoglobin); and Sicca (undiluted reduced hemoglobin), are studied against the background of hospital routine. They are examined as regards their working, and the possible errors are investigated with instructions as to how these can be avoided. In the Autenrieth method, the one-hour reading is used.

Heilmeyer's hemoglobin determination with the Stufen photometer has been somewhat modified, so that the laboratory apparatus customary in Sweden can be used.

Errors in reading, calculated on double determinations, are found to be practically equal in all three methods, i. e. approximately ± 0.55 % of the hemoglobin value obtained (average of three readings).

In the Sicca method, 1—2 hours' delay between the taking of the test and the reading is shown to raise the value obtained by approximately 2 %. The test should not, therefore, stand an unnecessarily long time before reading.

Time-studies are made. The total time for each hemoglobin determination is approximately the same for the three methods, when the interval between the taking of the test and the reading is not included. In hospital routine, however, the Sicca method takes nearly double the time for the laboratory nurse as compared with the Autenrieth method, since in the former she must herself do the majority of the cleaning.

Errors in the Method: = the error in an individual determination, calculated from the differences in double determinations, shows approximately the same order of magnitude in the three methods, i. e. approximately ± 2.0 % of the hemoglobin value obtained. The errors in the Autenrieth method are, however, with statistical probability lower than in the Sicca method. In a comparison of the differences between the hemoglobin values in Autenrieth/Stufen and Sicca/Stufen, no statistical difference is shown. This depends, in all possibility, on the fact that the so-called hematin error in the Autenrieth method plays a rôle. If the hematin error in the Autenrieth method is included, both methods are comparable, i. e. there is an error in the method of ± 2.3 %. This is on condition that the hemoglobin determination is made by competent laboratory technicians, and that in the Autenrieth method the one-hour reading-time is used, and the pipettes are controlled by weighing. The Stufen method is possibly somewhat preferable to the other two.

Standardization according to current use can result in an error of approximately ± 3.5 % of the hemoglobin value obtained. Different observers can give a variation of ± 3 %.

The error in the difference between two hemoglobin values is calculated for varying conditions of examination, and the probable limits for the existence of a true difference are tabulated.

The choice of method is discussed.

Literature.

Barkan, G. & Olesk, J.: Biochem. Zeitschrift 1937: 289: 251. — Barkan, G.: Lab. & Clin. Med. 1941: 1823. — Bierring, E.: Nord. Med. 1940: 6: 953. — Bonnier, G. & Tedin, O.: Biologisk Variationsanalys. 1940. — Ehrenberg, B.: Sv. Läkartidn. 1943: 35: 2134. — Enghoff, H.: Uppsala Universitets Årsskrift 1937: 9. — Heilmeyer, L. & Sunderman, A.: Deutsches Archiv f. klin. Med. 1936: 178: 397. — Heilmeyer, L. & Mutius, I.: Deutsches Archiv f. klin. Med. 1938: 182: 165. — Hesse, H. & Trier, M.: Ugeskr. f. Laeger 1937: 36: 935. — Hesse, H.: Acta Med. Scand. 1938: 97: 207. — Humperdinck, K.: Deutsches Archiv f. klin. Med. 1939: 183: 379. — Kaada, B.: Aesculap 1946: 1—2. — Sundberg, O.: Nord. Med. tidskrift 1938: 16: 1493. — Sundberg, O.: Sv. Läkartidn. 1945: 2: 57. — Sorensen, G.: Nord. Med. 1941: 10: 1117. — Sorensen, G.: Ugeskrift f. Laeger 1943: 8: 189. — Østerskov Jensen, K.: Ugeskrift f. Laeger 1943: 16: 402.

From the Medical Clinic of the Serafimer Hospital
and the State Bacteriological Laboratory, Stockholm.

Treatment of Experimental Tuberculosis in Mice and Guinea-pigs with Para-aminosalicylic Acid (PAS) and Streptomycin.¹

By

B. SWEDBERG and G. WIDSTRÖM.

(Submitted for publication September 11, 1947.)

Since 1946, when Lehman published his preliminary experiments, performed in vitro and clinically, regarding the bacteriostatic and disease-modifying effect of para-aminosalicylic acid (PAS), this preparation has been subjected to fairly extensive clinical tests at Swedish hospitals, while but a few animal experiments have been carried out. Originally, Lehman and his collaborators found PAS too toxic for guinea-pigs. Mice, on the other hand, stood it well. Their experiments, with an intraperitoneal infection of guinea-pigs acc. to Bernheim's method and simultaneous therapy per os and section after twelve days, were not very extensive. The weight of the omentum was accepted as an indicator of the degree of severity of the tuberculous process. In the course of these experiments the animals after a few days ate but little of the fodder containing PAS. The therapeutic experiment, for this reason, did not comprise more than about five days. They were able to ascertain an effect of PAS also in this brief experiment.

In comparatively extensive experiments with vaccination against tuberculosis the present authors have had much experience of guinea-pigs as test animals, acc. Widström's method, and, though on a lesser scale, of white mice. Both these types of animal

¹ Part of this paper was contributed to a discussion in Svenska Tuberkulosläkarföreningen (the Society of tb Specialists) on the 19th of February 1947.

can without any difficulty be infected intravenously, the guinea-pigs in the forelegs, the mice in the tail. In recent years mice have proved exceedingly useful test animals in determining the effect of streptomycin on tubercle bacilli in vivo (Youmans, and others).

In connection with vaccination experiments in the Autumn of 1946 the present authors began parallel therapeutic experiments with PAS against experimental tuberculosis in white mice. At the same time a great many determinations of resistance of strains of tubercle bacilli to PAS and streptomycin in Dubos' medium were obtained. These investigations are carried out partly in connection with clinical experiments that take place at the St. Göran's Hospital in Stockholm in cooperation with Dr. Westergren. Such observations as may emerge concerning the correlation between clinical developments and the fore-mentioned determinations of resistance will be published later.

In vitro experiments. Technique: to 5 ml of Dubos' medium (0.05 per cent of Tween, 0.5 per cent of albumin) are added 0.5 ml of falling dilutions of the bacteriostatic substance. For streptomycin the ratio of dilution has been 1/2, for PAS 1/5. The cultures that have been tested have previously been cultivated on Löwenstein substrate and then transferred to Dubos' medium. They have not been used until they have produced an abundant growth in three to five days. These cultures have served as mother cultures. After adding two drops of developed culture the experimental culture is subjected to daily observation. Such growth as may be noted is defined as + distinct growth, ++ moderate growth and +++ abundant growth, as compared with a constant scale of turbidity. In the records of the experiments the appearance of the tests has been stated at a time when the control culture has shown +++ (without any bacteriostatic admixture). These in vitro experiments (Table 1) disclosed, pro primo, that no sharp boundary exists between a concentration of PAS that arrests the growth of tubercle bacilli and one permitting growth, and, pro secundo, that the various strains vary, most considerably, in their primary susceptibility to PAS, much more than to streptomycin. In vitro the effect of PAS was found to be only relatively bacteriostatic. When the experiment was continued for an additional few days, growths of tubercle bacilli appeared in ever increasing concentrations of PAS.

Among strains of tubercle bacilli that proved primarily susceptible to PAS the well-known laboratory strain H 37 rv was

Table 1.

Difference in susceptibility to PAS (P) and streptomycin (M) between some strains of tubercle bacilli.

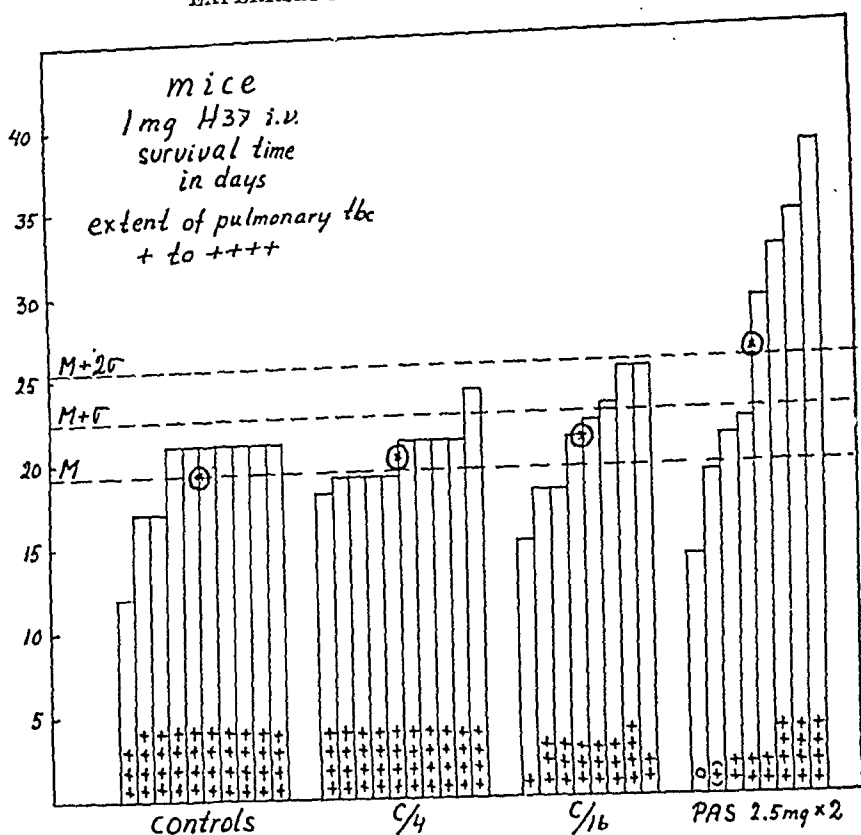
mg/100 ml PAS u/ml strepto- mycin.	10 5	0.4 1.25	0.016 0.31	0.0006 0.08	Control
H 37 P	(+)	(+)	+	++	+++
M	—	—	—	+	+++
2101 P	—	—	+	+(+)	+++
M	—	—	—	—	+++
Ravenel P	++	++	+++	+++	+++
M	—	—	—	—	—
K 147 P	++	++	++	++	+++
M	—	—	—	—	—
O 17 P	+++	+++	+++	+++	+++
M	—	—	+	+++	+++
S 102 P	++(+)	++(+)	++(+)	+++	+++
M	—	—	++(+)	++	+++
S 120 P	+++	+++	+++	+++	+++
M	not tested	(+)	++(+)	++(+)	+++

noted and among the more resistant ones the likewise well-known Ravenel strain.

Animal experiments. In our preliminary animal experiments with PAS-treatment of experimental tuberculosis in white mice (Fig. 1), we used the PAS-susceptible H 37 strain in comparatively large dosages, viz., 1 mg intravenously. In addition series of mice were infected with 1/4 and 1/16 dosages in order to serve as a standard for calculating the order of magnitude of a possible effect in such groups of animals as were subjected to therapeutic experiments. PAS was added by means of subcutaneous injections of 2.5 mg twice daily in mice of about 20 grammes, corresponding to a clinical dose of 14 grammes per os for an adult patient.

The time of survival of the animals served as a measure of the degree of severity of the infection or of the therapeutic result, if any. The macroscopically judged lung findings (0 to +++) were recorded.

In this initial experiment all control animals as well as the animals of the standard groups died in massive tuberculosis (except two animals with 1/16 dosage where the extent of the pulmonary tuberculosis was somewhat more restricted). Also in



In a preliminary experiment guinea-pigs were seen to endure 25 mg PAS \times 2 subcutaneously without showing any objective morbid symptoms. In a new experimental series, therefore, both mice and guinea-pigs were used, the effect of PAS in varying dosages being compared with that of, *inter alia*, streptomycin. Further, the possible combined effect of PAS and streptomycin was investigated.

This time in the experiments on mice the PAS-susceptible strain H 37 as well as the relatively PAS-resistant Ravenel strain were used. Fig. 2 reveals a marked increase on this occasion of the time of survival in the standard groups in the experiment with H 37 (the infection dose this time being only 0.06 mg in the

control as well as therapy series) but the effect of PAS 2.5 mg \times 2 subcutaneously (PAS I) or of half this dosage (PAS II) is not statistically significant. Streptomycin in a dosage of 500 units \times 2

Table 2.

Macroscopic post-mortem findings in guinea pigs from control and therapeutic groups in chemotherapeutic experiments (same experiment as in fig. 4).

	Survival time, days	Macroscopic post-mortem findings (extent of tbc)		
		lungs	liver	spleen
a) control and standard groups.				
Control group	19	+	++	+++
	20	+(+)	++	+++
	20	+(+)	++(+)	+++
	21	+(+)	+++	++++
	21	++	+++	++++
	22	0	0	+++
	22	(+)	+(+)	+++
	22	++	+++	++++
	29	++(+)	+(+)	++++
	32	+++(+)	++	+++(+)
	Mean 22.8			
	Mean $\pm 2 \sigma = 31.54$			
Standard group $\frac{1}{25}$ dose	33	+(+)	++(+)	+++
	34	+(+)	++++	+++
	35	+(+)	++	++++
	36	+(+)	+++	+++(+)
	36	++(+)	++++	+++(+)
	39	++	++(+)	++++
	42	++	++++(+)	++++
	44	++(+)	++++	+++(+)
	46	+++	+++(+)	+++(+)
	58	++(+)	++(+)	+++(+)
	Mean 40.1			
	b) PAS-treated groups.			
PAS 20 mg \times 2 s. c. (PAS I)	15	+(+)	++(+)	+++
	17	0	0	0
	17	0	0	+
	21	++	+++	++++
	23	+(+)	++	+++
	25	++	+++	+++(+)
	32	++(+)	+(+)	+++(+)
	32	++(+)	++	+++(+)
	34	++(+)	+(+)	++++
	39	++++	+++	+++
	Mean 25.5			

	Survival time, days	Macroscopic post-mortem findings (extent of tbc)		
		lungs	liver	spleen
PAS 10 mg \times 2 s. v. (PAS II)	20	0	++	+++(+)
	21	0	0	+++
	25	+	++	+++
	25	++	++(+)	++++
	25	+(+)	0	++++
	25	++(+)	++++	++++
	27	++	+++	++++
	29	++	++++	++++
	29	++(+)	++++	++++
	30	++(+)	++++	+++
	Mean 25.6			
c) Streptomycin-treated groups after 36 days one dose daily. Treatment stopped after 112 days.				
Streptomycin 5000 u \times 2 s. c. (SM I)	87	0	0	0
	127	0	0	++
	135	+(+)	++ (+)	+++(+)
	137	++	++	++
	142	+(+)	++	++
	146	++(+)	++	+++(+)
	155	++(+)	++	+++(+)
	158	+(+)	0	+++
	165	+(+)	++	++
	(Killed) 205	0	0	0
	Mean 145.7			
Streptomycin 2500 u \times 2 s. c. (Sm II)	25	+	++	0
	53	0	0	+++(+)
	64	++(+)	+++	+++
	85	0	0	0
	93	+	0	0
	96	++(+)	+++(+)	+++(+)
	102	+(+)	++	+
	107	++	++(+)	+(+)
	107	+++(+)	++	+++
	114	+++	+++	+++
	Mean 84.6			
d) Combined PAS—streptomycin—treated groups after 36 days one dose daily. Treatment stopped after 112 days.				
PAS 10 mg \times 2 s. c. + streptomycin 2500 u \times 2 s. c. (PAS II + SM II)	41	0	0	0
	46	+	0	0
	53	+	+++(+)	++
	58	++	++(+)	++
	68	+	++(+)	+++(+)
	71	+	++++	+++
	73	0	+++	+
	79	+(+)	0	++
	114	0	0	0
	128	+++	+++	+++
	Mean 73.1			

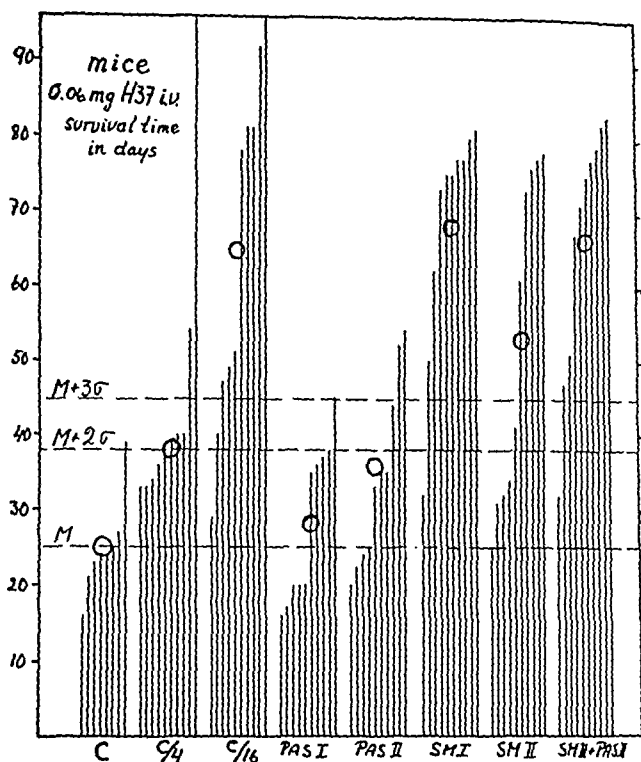


Fig. 2.

subcutaneously (SM I) and in half this dosage both disclosed considerable effect. PAS and streptomycin in combined half-dose (PAS II + SM II) caused a certain, though not statistically significant increase in the time of survival. After 36 days only one injection daily was administered in any of the experiments. After 112 days the treatment was entirely discontinued.

Fig. 3 demonstrates a therapeutic experiment with the same PAS and streptomycin doses (PAS I and SM II) as in Fig. 2, though with the more resistant Ravenel strain as infection, *i. e.* 0.06 mg intravenously. Also in this instance the time of survival increases in the standard groups. The effect of PAS is not definite, while that of streptomycin is considerable.

In a more extensive experiment with guinea-pigs (Fig. 4) the Ravenel strain was used. 2 mill. tubercle bacilli were administered intravenously in the forelegs of the control and therapy groups. Standard groups of 1/5 and 1/25 dosages show the effect on the time of survival of a decrease in the doses. Also in this case the

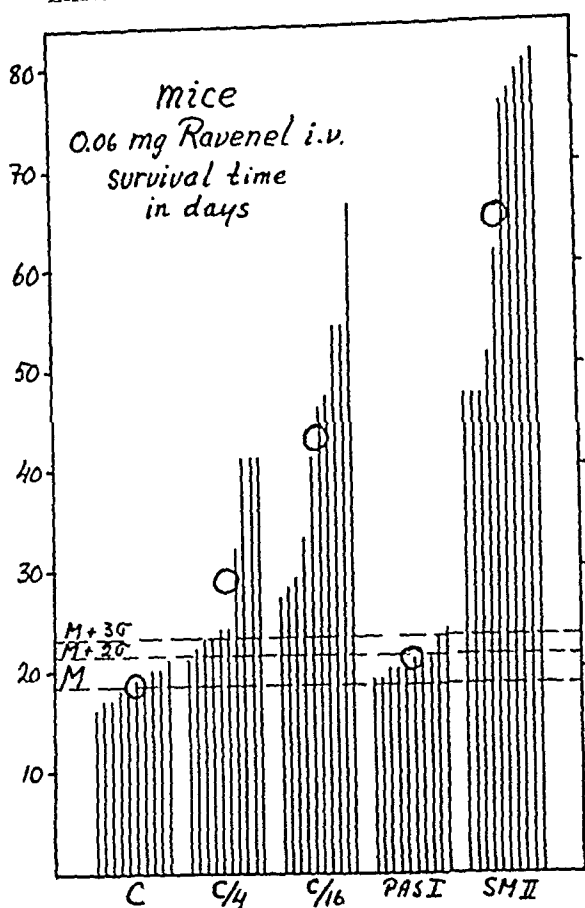


Fig. 3.

time of survival served as a measure of the degree of severity of the infection as well as of the therapeutic result, if any. The macroscopic post mortem findings served as a control. Table 2 a—d shows details from guinea-pig groups treated with PAS and streptomycin. PAS of 20 g \times 2 subcutaneously (PAS I and half this dosage, PAS II), corresponding to a full clinical dose (14 grammes per os to adults) has no definite effect. Sodium salicylate (NS), in doses corresponding to those of PAS, discloses no therapeutic nor any definitely toxic effect. Promizole (Diason Astra, Pr) 0.07 grammes \times 2 per os has a definite, though not particularly pronounced effect. On the other hand, streptomycin in a dosage of 5,000 units \times 2 subcutaneously (SM I) and half this dosage (SM II) shows a considerable effect. As against half the streptomycin dose only, a combined dose of half PAS and half streptomycin (PAS II + SM II) causes no increased therapeutic

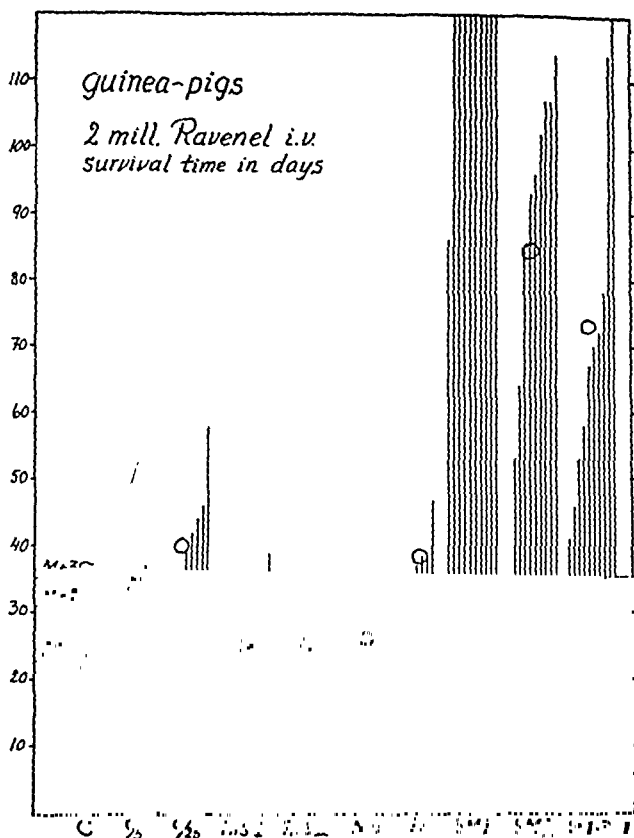


Fig. 4.

effect. Also in these experiments, after 36 days only one injection daily was given, after 112 days none.

One of the disadvantages of streptomycin is the relatively easy development of chemoresistance. In order to investigate whether the same applies to PAS determinations of the resistance to PAS and streptomycin of the H 37 and Ravenel strains were made in vitro, from ordinary laboratory strains, as well as from strains cultivated from organs of animals treated with PAS.

From Tables 3—7 it will be seen that no definite increase or decrease in susceptibility or resistance to PAS has occurred under this treatment. In the one single case where the resistance of a strain from an animal treated with a combination of PAS and streptomycin has been tested (Table 7), no resistance to either PAS or streptomycin could be ascertained.

While the experiments took place, Youmans published thera-

peutic experiments with PAS against an experimental tuberculosis in white mice, stating that PAS is »at least moderately effective for the suppression of tuberculous infection in white mice, though not so effective, in the doses, used as streptomycin». Thus, we are in a position to confirm these results of Youmans.

Table 3.

In vitro test for susceptibility to PAS (P) and streptomycin (M) before and after treatment (PAS full dose of H 37 in mice experiment.

Mg/100 ml PAS u/ml strepto- mycin	10 5	0.4 1.25	0.016 0.31	0.0006 0.08	Control
H 37 untreated P M	— —	(+) —	+	++	+++ +++
H 37 PAS- P treated 29 d. M	(+) —	(+) —	+	++	+++ +++
H 37 PAS- P treated 34 d. M	(+) —	(+) —	+	++	+++ +++
H 37 PAS- P treated 34 d. M	(+) —	(+) —	+	++	+++ +++

Table 4.

In vitro tests for suscepibility to PAS (P) and streptomycin (M) before and after treatment (PAS full dose) of Ravenel in mice experiment.

mg/100 ml PAS u/ml strepto- mycin	10 5	0.4 1.25	0.016 0.31	0.0006 0.08	Control
Ravenel, P untreated M	++(+) not tested	+++ —	+++ +(+)	+++(+) +++	+++(+) +++(+)
Revenel, PAS- P treated 21 d. M	++(+) not tested	++(+) (+)	++(+) +(+)	++(+) ++(+)	+++ +++
Ravenel, PAS- P treated 21 d. M	++(+) not tested	+++ —	+++ +(+)	+++(+)+ +++	+++(+) +++(+)

Table 5.

In vitro tests for susceptibility to PAS (P) and streptomycin (M) before and after treatment (PAS full dose) of Ravenel in guinea-pig experiment.

mg/100 ml PAS u/ml strepto- mycin	10 5	0.4 1.25	0.016 0.31	0.0006 0.08	Control
Ravenel, P untreated M	++(+) —	++(+) —	++(+) (+)	++(+) ++(+)	+++(+) +++(+)
Ravenel, PAS- P treated 32 d. M	++ not tested	++(+) +	++(+) ++(+)	+++ ++(+)	+++ +++
Ravenel, PAS- P treated 32 d. M	++(+) not tested	++ (+)	++ ++(+)	++(+) ++	+++ +++
Ravenel, PAS- P treated 34 d. M	++(+) —	++ —	++(+) —	++(+) ++	+++ +++

Table 6.

In vitro tests for susceptibility to PAS (P) and streptomycin (M) before and after treatment (PAS, half dose) of Ravenel in guinea-pig experiment.

mg/100 ml PAS u/ml strepto- mycin	10 5	0.4 1.25	0.016 0.31	0.0006 0.08	Control
Ravenel P untreated M	++(+) not tested	++(+) —	++(+) (+)	++(+) ++(+)	+++(+) +++(+)
Ravenel PAS- P treated 25 d. M	++(+) not tested	++(+) —	++(+) (+)	++(+) ++(+)	+++ +++
Ravenel, PAS- P treated 27 d. M	++ not tested	++ ++	++(+) ++(+)	++(+) ++(+)	+++ +++
Ravenel, PAS- P treated 29 d. M	++(+) not tested	++ (+)	++ (+)	++ ++	+++ +++

Table 7.

In vitro tests for susceptibility to PAS (R) and streptomycin (M) before and after treatment. (PAS and streptomycin, half doses combined) of Ravenel in guinea-pig experiment.

mg/100 ml PAS u/ml strepto- mycin		10	0.4	0.016	0.0006	Control
		5	1.25	0.31	0.08	
Ravenel, untreated	P	++(+)	+++	+++	++(+)	+++(+)
	M	not tested	—	+(+)	+++	+++(+)
Ravenel, combined treated 79 d.	P	++(+)	+++	+++	+++	+++(+)
	M	not tested	(+)	+	++(+)	+++(+)

Summary.

In a series of *in vitro* experiments a considerable difference in primary susceptibility to PAS in different strains of tubercle bacilli is ascertained.

In animal experiments with PAS mice as well as guinea-pigs can be used. A certain effect of PAS *in vivo* on the strain H 37 with its susceptibility to PAS can be noted. This effect is not always statistically significant. In one experiment PAS-treatment has an effect equal to a reduction of the infection dosage to 1/16, but in two other experiments the effect falls below that obtained through a reduction of the infection dose to 1/4—1/5. No effect of PAS on an infection with the PAS-resistant Ravenel strain has proved ascertainable. In corresponding animal experiments streptomycin discloses a considerable effect on both strains.

In these experiments a combined treatment with PAS and streptomycin has caused no ascertainable effect over and above that of each drug separately, whether in the strain susceptible to PAS or in that resistant to it.

In the *in vitro* experiments no increase either in susceptibility or resistance to PAS, acquired in the course of the treatment, has been demonstrated.

Literature.

Lehman: *Lancet*, Jan. 5, 1946, p. 15. — Lehman: *Svenska Läkartidningen* 43: 2029, 1946. — Swedberg: *Nord. Med.* 1947, 36: 2149, 1946. — Widström & Swedberg: *Nord. Med.* 1947, 36: 2148, 1946. — Widström: *Acta Tbc. Scand.* XX: 2—4: 171, 1947. — Youmans: *Quarterly Bull. N. W. Univ. Med. School* 20: 4: 420, 1946. — Youmans & McCarter: *Am. Rev. tbc.* 52: 432, 1945. — Youmans, Williston, Feldman & Hinshaw: *Proc. staff meet.*, Mayo Clin. 1946.

From the Blegdam Hospital, Copenhagen
(Chief: Professor H. C. A. Lassen, M. D.) and
The State Serum Institute, Copenhagen
(Director: J. Ørskov, M. D.).

Specific Serum Treatment of Pfeiffer Meningitis.¹

By

KNUD BRØCHNER-MORTENSEN, HANS CHR. ENGBÆK
and KAI SCHMITH.

(Submitted for publication August 4, 1947.)

The introduction of sulphonamide and penicillin treatment has very decidedly modified the prognosis of purulent meningococcic and pneumococcic meningitis, whereas on Pfeiffer meningitis the effect of these drugs has varied considerably and in most cases has been very small. In the United States, however, good and constant results have been observed in recent years from a combination of chemotherapy and the administration of type-specific rabbit immune serum.

Pfeiffer's bacillus, *Haemophilus influenzae*, was isolated by Pfeiffer in 1892, and the first descriptions of this microbe as a cause of purulent meningitis were given by Fraenkel (1898) and Slawyk (1899).

In 1930—33 Pittman (35, 36, 37) demonstrated that Pfeiffer's bacillus occurs in both non-capsulate and capsulate forms. According to their capsule antigens the capsulate forms are differentiated into six types, a, b, c, d, e and f, and Pittman showed that the great majority of the strains isolated from purulent spinal fluids in the United States are capsulate and of Type b.

As in the case of the pneumococcus, the specific substances in the capsule are various polysaccharides, most of which have been isolated and studied by Heidelberger et al. at the Columbia University (10, 11, 28).

¹ Carried out with support from P. Carl Peterson Foundation.

Pfeiffer meningitis is essentially a disease of early childhood and particularly frequent in the first three years of life. It occurs somewhat more often in winter than in summer, and in Denmark there is some tendency towards an accumulation in the final quarter of the year (18). The disease is never epidemic.

The *clinical picture* of Pfeiffer meningitis is subject to a good deal of variation, from a fulminating, acute course as in severe meningococcic meningitis to a more insidious one as in tuberculous meningitis. The tendency perhaps is mostly towards a protracted course.

In older children (and in the rare instances when the disease attacks adults) the typical meningeal symptoms are quick to make their appearance. Petechiae, ecchymosis and exanthema are never seen.

In most cases the general condition rapidly becomes very bad, but in some cases the patients are relatively little affected at first.

The temperature is high as a rule, sometimes irregular high and fluctuating giving place to other periods in which the temperature is lower.

After some time there are occasional symptoms of complicating encephalitis or neuritis.

In some cases the meningitis is complicated with other purulent processes such as otitis, sinusitis, pneumonia, empyema, arthritis and subcutaneous or intramuscular abscesses; but generally it is difficult to decide whether the meningitis is the primary pathological process from which the bacteria have subsequently spread to other parts of the organism, or it is secondary for example to otogenous or nasal affections.

With infant children the diagnosis is often difficult, as the direct symptoms of meningeal affection in most cases are but little pronounced in the early stage. Frequently the first objective sign of meningitis will be a tense fontanelle. Rigidity of the back of the neck and of the spine, Kernig's and Brudzinski's symptoms are often absent or only faintly developed and are difficult to appraise. For these reasons it is important always to make a spinal puncture in children who are feverish from an unknown cause.

The analysis of the spinal fluid generally shows numerous polynuclear leucocytes and a moderate protein increase. The sugar and chloride contents are usually reduced.

The conclusive diagnosis of Pfeiffer meningitis is arrived at

with the finding of Pfeiffer's bacillus. In some cases it is seen by direct microscopy, particularly after centrifuging, but in many cases the bacterium is only found by cultivation, and therefore the spinal fluid should always be examined in a special bacteriological laboratory.

Prognosis: The spontaneous lethality of Pfeiffer meningitis varies between 90 and 100 %, all age groups considered. For children under two years it is practically 100 % (7).

The results from treating with sulphonamide vary considerably in different materials, but most frequently 30 to 70 % of children older than seven months will die, and almost all younger children (1, 12, 16, 17, 21, 22, 24, 26, 30, 38, 40).

In Denmark in a material comprising 93 patients under sulphonamide treatment there was a lethality of 82 among children older than 7 months. All below 18 months died. Of other 27 patients treated with sulphonamide and penicillin combined, 26 died (18).

At one time horse immune serum was used, without definite effect (19, 24, 26, 29, 37, 44).

Events took a decisive turn, however, when Alexander (2) in 1939 inaugurated treatment with type-specific rabbit immune serum, later combined with sulphonamide. Treating 26 patients with serum alone Alexander found a lethality of 35 % (3). In her last material of 87 patients (5) treated with sulphonamide and serum combined the lethality was 22 %, and in various other materials it varies between 7 and 24 (15, 25, 38, 39, 42). But the lethality is still high (getting up to 80) among children under 7 months.

Very promising reports have most recently appeared (6, 8, 9, 13, 27, 31, 32, 33, 43) concerning the effect of streptomycin, and the lethality seems to be about the same as when type-specific rabbit immune serum is used. It is therefore the advice of various authors (9, 27, 31), especially in the severe acute and sub-chronic cases, to treat at once with both streptomycin and sulphonamide as well as type-specific rabbit immune serum, by which means the lethality can, it is held, be reduced still more.

Own Observations.

In Denmark so far the examination of capsule antigens in strains of various origins has led to the identification of five

different types. Of 126 strains cultivated from purulent spinal fluids, 125 were of the same serological type and identical with the American Type b.¹ Type a was found in only one instance.

In the period from October 1946 to February 1947 at the Blegdam Hospital in Copenhagen we have given 7 patients with Pfeiffer meningitis (Type b) a combined treatment with sulphonamide and type-specific rabbit immune serum. The first patient received serum prepared by E. R. Squibbs & Sons,¹ the others serum produced at the State Serum Institute, Copenhagen. The specific components of the latter serum were concentrated according to the same principle as that applied in the manufacture of concentrated pneumococcus serum. The strength of the serum is measured in mg specific antibody-nitrogen and is also indicated in units (1 mg = 1,000 units). Animal tests have shown the effect to be type-specific.

Of these seven patients, two died. These two were 7 and 9 months old, the others were 5 months, 12 months, 18 months, 18 months and 5 years.

One of the two patients who died (No. 3) had been treated for severe mastoiditis. The spinal fluid had originally been normal, but numerous polynuclear cells were found later, and on cultivation, one colony of Pfeiffer bacilli, Type b, was found once. The autopsy revealed no definite sign of meningitis. In all probability the patient died as the result of severe mastoiditis, and as serum was not given until the child was moribund, the case tells us nothing of the effect of the serum treatment.

When working out the principles for the treatment we proceeded along the lines recommended by Alexander, adapted to the laboratory possibilities available. At times the treatment was marked by the small quantity of concentrated serum at our disposal.

Although our present material is very small, we believe we can single out certain points of importance.

As the intravenous injection of serum in babies is often very difficult, we soon decided to administer it intraperitoneally, it having been demonstrated (14) that this application ensures quicker absorption and higher concentration than intramuscular injection. We observed no discomfort from this procedure.

¹ We are obliged to Dr. H. E. Alexander, Babies Hospital, New York, for furnishing us with type-specific strains and test sera, and to Dr. A. Holm, E. R. Squibbs & Sons, New Jersey, for therapeutic serum.

It seems to be very important to begin the specific treatment at the earliest possible moment and to give fairly large doses of antibody immediately.

For Patient No. 2 the diagnosis was made very quickly and a vigorous specific treatment was commenced only 24 hours after the onset of the disease. Complete cure was obtained in a very short time. However, one contributory factor was possibly the circumstance that the patient was a relatively old child (5 years). As already stated, prognosis is best for older children.

While the treatment is in progress the temperature curve will be of only little guidance. In the case of one of those who died (No. 5) there was an initial fall in the temperature, decreasing cell count and transient improvement. As the patient was only 7 months old, was very ill and the onset of the disease was at least six days before, a more intensive treatment ought to have been instituted from the start, but this was impracticable owing to lack of serum. The rapidly falling titre for free antibody in the blood shows that the treatment was insufficient. It is also possible that serum should have been administered intraspinally.

In respect of Patient No. 4, for whom the bacteriological diagnosis was made somewhat late owing to the slow growth of the bacteria, a distinct improvement was observed in the general condition under the sulphonamide and penicillin treatment, the temperature became normal and the pleocytosis subsided, although bacteria were still found on cultivation. However there was a rapid relapse, and a quick cure was only effected after the application of a large quantity of antibody.

On the other hand, a persistent temperature increase may be a result either of the sulphonamide therapy (drug fever) or the administration of antibody (serum reaction). This was undoubtedly the case with Patient No. 1 and probably also Patient No. 6. In the latter instance, however, we considered it necessary to prolong the treatment because bacteria appeared twice in the spinal fluid after it had been sterile. In all the patient was given 770 ml serum = 1,071 mg specific antibody-N divided over 14 injections spread over a period of 33 days. Neither in this nor in the other cases did we observe symptoms of any gravity attributable to the administration of non-specific protein. As a general rule, however, it will undoubtedly be better to apply large quantities of serum at the very beginning rather than continue administration over such a long time.

Alexander attaches great importance to the spinal-sugar values in determining both the dosing and the specific treatment. For values under 15 mg % Alexander gives 100 mg antibody-N, between 15 and 25 mg % she gives 75 mg, between 25 and 40 mg % 50 mg and over 40 mg % 25 mg.

The value of the spinal sugar prior to the commencement of treatment is also stated to have a bearing on the prognosis, it being very grave at values below 15 mg % and good at values over 30 mg % (7).

With our small experience of course we are unable to reach any final conclusions, but we must point out that for our two dead patients the lowest observed values for the spinal sugar were 37 and 15 mg %, whereas for those who survived they were 10, 43, 11, 4 and 23 mg %.

Our preliminary view is that there is a risk of underdosing by following Alexander's dosage scheme on the basis of the spinal sugar values.

Another guide to the treatment is the presence of free antibody in the patient's serum. Alexander requires an excess still demonstrable in a serum dilution of 10. With our technique the antibody titre in most cases is no higher than 4, in some instances 8, which in our present experience seems to be adequate.

We have said that our experience so far is small, but we believe it to be important to present the material now, in order to draw attention to the possibility of obtaining better results than hitherto in the treatment of patients with Pfeiffer meningitis.

We believe it advisable always, and as early as possible, to give two large serum doses of 100 mg antibody-N, the one 24 hours after the other — according to the same principle observed in the pneumococcus serum treatment. The serum should be administered intravenously, or in the case of small children intraperitoneally. Raise a fold of the peritoneal skin and inject in the medial line about 2 cm below the umbilicus with a bevelled needle.

Six to twelve hours after the last serum injection the quantity of free antibody in the patient's serum should be determined and the continued serum treatment dosed accordingly. In addition, the spinal fluid should be tested daily (cell count — spinal sugar — bacteriological test) as long the disease is still thought to be out of control.

If bacteria continue to appear in the spinal fluid several days

after the serum treatment is begun, it is probably advisable to inject serum intraspinally, about 10—15 mg antibody-N, after removing a suitable quantity of spinal fluid.

The serum treatment should be accompanied simultaneously with sulphonamide (Sulphathiazol or Sulphadiazine) according to the usual principle in large doses.

This treatment should be commenced as soon as the diagnosis of purulent meningitis is made. Treatment with penicillin has no definite effect.

If streptomycin is obtainable it should be used as well — applied according to American experience in intermittent intramuscular injections every three hours, in all 0.6 to 1 g daily.

Treatment with chemotherapeutica must be continued for about a week after the spinal fluid is found to be sterile.

None of these patients were treated with streptomycin. Since february 1947 we have treated 14 patients with Streptomycin, Sulphonamide and Serum. The results will be published later.

Patient No. 1. ♂ S. 5 months. Hosp. 28. 9. 46—9. 11. 46. No. 6049/46. Previously well.

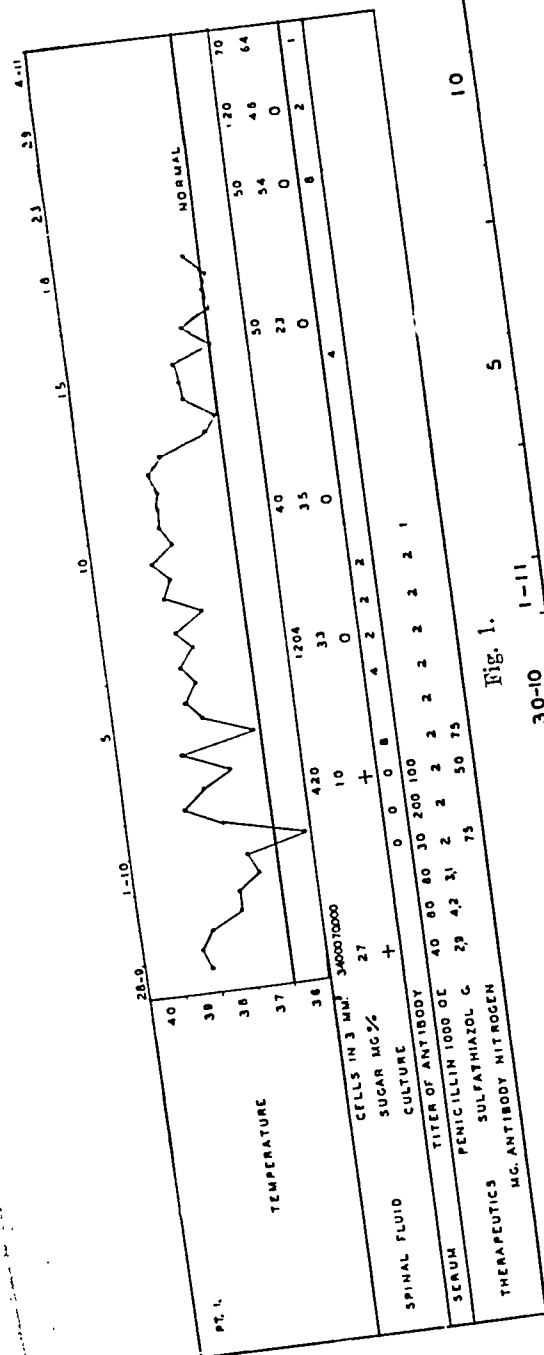
For 10 days watery faeces several times daily and occasional vomiting. During last few days tp. rose to 40°; increasingly restless. On admission tp. 39.1. Very ill, with tense fontanelle and rigid neck. Numerous polynuclear cells found in spinal fluid, wherefore penicillin-sulphonamide treatment instituted. Tp. fell and general condition improved slightly. On 1. 10. 46 Serum. Inst. reported finding of Pfeiffer bacilli Type b in culture from spinal fluid taken 28. 9. 46 and type-specific antibody (rabbit serum Squibb) was given intravenously and intramuscularly. Pfeiffer bac. still in sp. fluid on 3. 10. 46, but afterwards it is sterile.

Tp. remains high but general condition improves steadily and cell count falls. After discontinuing sulphonamide and after slight exanthema on 12.—13. 10. 46 resembling serum exanthema, tp. falls. General condition then improves quickly, but cell count still somewhat increased. Discharged well on 9. 11. 46, and at control examination on 13. 1. 47 the child found to be quite natural. Regarding details of laboratory finds and treatment, see fig. 1.

Patient No. 2. ♀ H. 5 years. Hosp. 30. 10. 46—19. 11. 46. No. 6948/46. Previously well.

The day before admission the child comes home crying and complains of receiving a blow on the head while playing. Falls asleep and is difficult to wake. Later vomiting, shivering and teeth-grinding.

Tp. on admission 39°, very ill, drowsy. Pronounced neck and back rigidity. Numerous polynuclear cells and Pfeiffer bacilli Type b in spinal



10

Fig. 1.

30-10 1-11

PT. 2.

TEMPERATURE

CELLS IN 3 MM.

SUGAR MG%

SPINAL FLUID

SERUM

THERAPEUTICS

MG. ANTIBODY NITROGEN

Fig. 2.

fluid, wherefore chemotherapy and specific serum treatment (intraven.) instituted immediately. Tp. falls next day and spinal fluid sterile, and on the following day the child is almost unaffected. Five days later all symptoms subsided, and on the 21st day she is discharged perfectly well.

For details of laboratory finds and treatment, see fig. 2.

No serum exanthema observed.

Patient No. 3. ♀ A. 9 months. Hosp. 5. 11. 46—† 10. 11. 46. No. 7132/46. Previously well.

For 8—10 days serous nasal catarrh, decreasing in intensity. Day prior to admission tp. 39.6—40.5°.

Tp. on admission 40.5, and throughout the observation time remains between 40 and 41°. Very little affected on admission and neck rigidity is doubtful. Spinal fluid contains 5/3 cells. Otoscopy reveals bilateral Otitis media, wherefore bilateral myringotomy performed at once and three days later Resectio proc. mast. Otitis being found on both sides.

Despite intensive treatment with penicillin and sulphonamide the child sank quickly. On 7. 11. 46 there were numerous polynuclear cells in the spinal fluid, and one colony of Pfeiffer bacilli Type b was found in culture. On 8. 11. 46 the cell count was still higher, but no bacteria (though no serum given yet).

Immediately Pfeiffer bacilli were found by culture from spinal fluid the patient was given type-specific antibody (intraperitoneally) In this stage the child was moribund and death occurred 21 hours later.

Autopsy showed the meninges perhaps slightly hyperaemic, but no thickening, nor was fibrine or pus observed. The membranes were almost clear and quite transparent.

The cerebral tissue was moist, but without haemorrhage or abscesses.

Thus no meningitis could be found by autopsy (Bj. Wimtrup).

Patient No. 4. ♀ S. 18 months. Hosp. 20. 11. 46—14. 12. 46. No. 7478/46.

Nasal catarrh and a cough for 9—10 days, treated with in all 5 g sulphonamide. Admitted to children's hospital, where spinal puncture revealed 3,000/3 cells, wherefore she was transferred immediately to Blegdam Hosp. On admission there tp. was 39.4°. Very ill, pale-cyanotic, neck rigid, and diffuse rales over both lungs. During treatment with penicillin and sulphonamide the tp. fell and the pleocytosis decreased. On the 5th day tp. rose again, which was regarded as drug fever, and after suspending the sulphonamide the tp. fell to 37°, patient being quite well.

Although Pfeiffer bacilli Type b were found in the spinal fluid taken on 23. 11. 46, no specific serum treatment was given under these circumstances.

Next day, however, tp. rose again and patient again became ill, so serum (intraperitoneally) as well as penicillin and sulphonamide were administered.

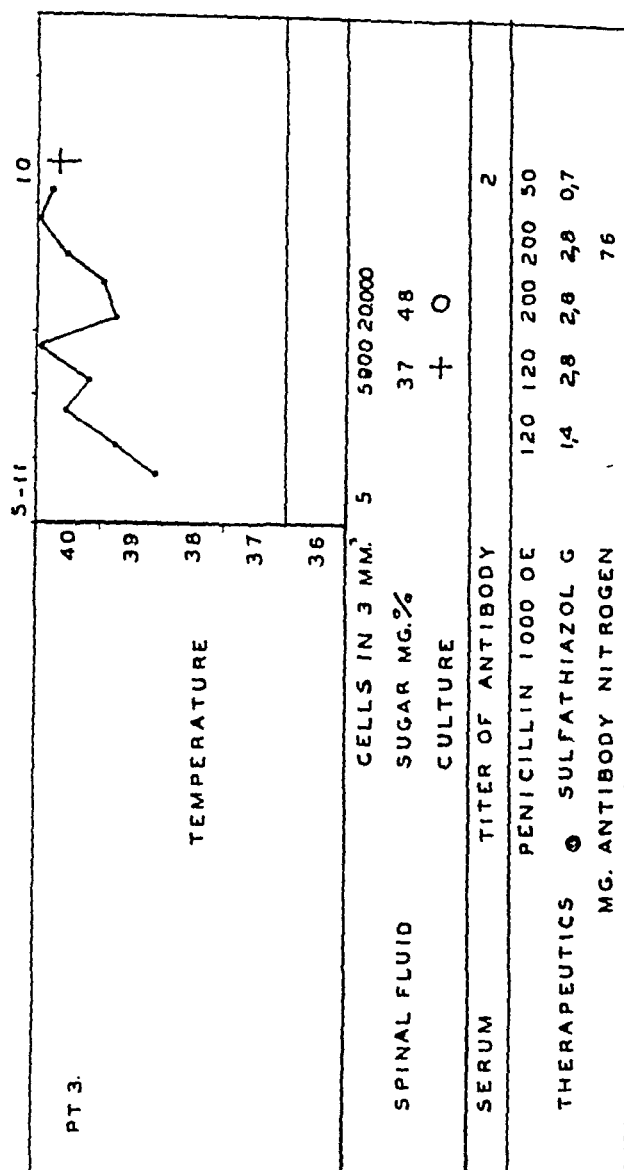


Fig. 3.

On the following day the spinal fluid was sterile. Tp. fell to and remained at normal values, and the patient was doing very well in a few days. Nine days after the serum application there was serum exanthema without tp. reaction. On the 25th day patient was discharged as cured.

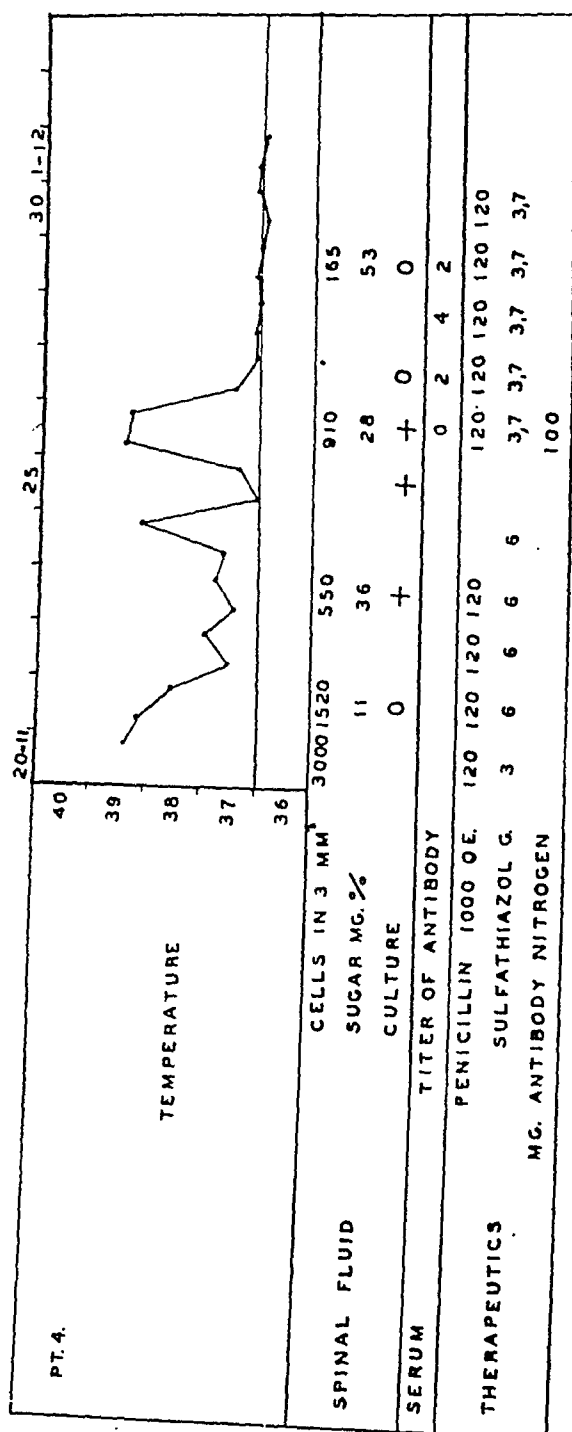


Fig. 4.

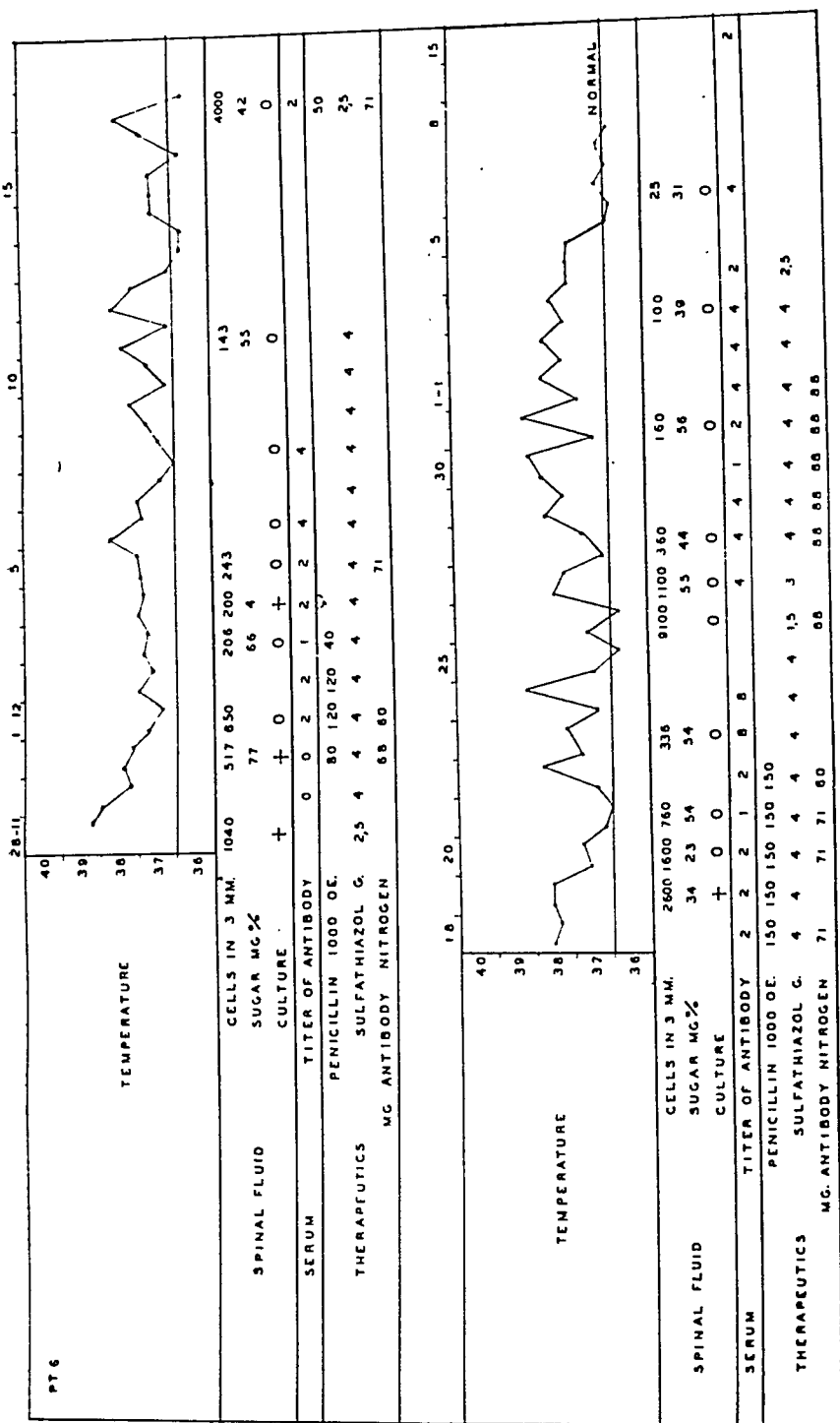


Fig. 6.

Patient No. 5. ♀ C. 7 months. Hosp. 28. 11. 46—† 10. 12. 46. No. 7701/46. Six days before admission tp. 40.5°, swelling and redness around right eye. The swelling subsided in two days, but the tp. continued high.

Tp. on admission 40°; very ill, with tense fontanella and pronounced neck rigidity; no petechiae.

Numerous polynuclear cells and Pfeiffer bacilli, Type b, in spinal fluid, wherefore treatment instituted as shown in fig. 5. Serum intraperitoneally, in the last two days also intraspinally. Patient improved temporarily in first 24 hours. On 7th day spinal fluid sterile, but with bacteria on following day. On 8th day urticarial exanthema like serum exanthema, but as no reaction occurred after injection of small quantity serum intramuscularly, treatment continued and the exanthema disappeared next day. Explorative myringotomy performed several times during attack without revealing pus.

General condition weakened gradually and death occurred on 13th day. Autopsy revealed severe meningeal changes.

Patient No. 6. ♂ K. 12 months. Hosp. 28. 11. 46—30. 1. 47. No. 7685/46. August 1946 Otitis media. Otherwise always well previously.

Four days before admission tp. high with temporarily fall after sulphonamide. During last day drowsy, wailing; vomited once.

On admission tp. 39.2°. Ill, with back and neck rigidity. Spinal fluid contains numerous polynuclear cells and bacteria which culture proved to be Pfeiffer bacilli Type b.

In response to sulphonamide, penicillin and later type-specific antibody, tp. fell, general condition improved, and spinal fluid became sterile with falling cell count.

However, some days later bacilli appeared again and tp. rose, wherefore serum again administered. The Bacilli disappeared immediately, to reappear 14 days later with simultaneous tp. rise, and pleocytosis increases. Injection of very large serum doses now continued (intraperitoneally, on 28. 12. 46 also intraspinally), despite transient serum exanthema on 19. 12. 46. Thereafter spinal fluid sterile.

On about 26. 12. 46 the child very ill. Cell count rose to 9100/3, but the condition improved gradually, tp. fell and pleocytosis decreased. On 31. 1. 47 the child discharged in perfectly good condition without psychic or somatic defects.

Patient No. 7. ♀ R. 18 months. Hosp. 30. 1. 47—27. 2. 47. No. 761—47.

Four days before admission suddenly ill with cold shiverings, feverish, vomiting and drowsiness.

Tp. on admission 38.6°, very ill, with marked neck rigidity but no petechiae.

Spinal fluid contained numerous polynuclear cells, and cultivation revealed Pfeiffer bacilli Type b, wherefore treatment instituted as in fig. 7. Serum intraperitoneally.

It being the general rule that the few streptomycin-resistant bacteria in an otherwise sensitive culture are sensitive to sulphonamides, treatment with agents of this group will slow down the rate of multiplication of the streptomycin-resistant bacteria and thus possibly prevent the development of resistant strains.

Working on this theory Alexander & Leidy (1947) (4) tried treating the severe cases of meningitis with streptomycin and sulphadiazine alone. So far, however, the material is only small.

In their latest work (4) these two authors advise the administration of streptomycin in doses of 44 mg per day kg body weight. The injections should be given intramuscularly every three hours, in addition to once a day intrathecally, 25 mg for children under three years, 50 mg for older ones. The first two injections may be given with an interval of 12 hours if necessary. To avoid the serious toxic symptoms, the streptomycin treatment should not be continued longer than four or five days. What is not achieved by this treatment will scarcely be achieved by longer dosing.

It might have been anticipated that with the intensive treating of Pfeiffer meningitis many of the serious cases which now survive would manifest signs of deep, irreparable cortical changes and hydrocephalus. To judge from the literature, this is not the case, even if there are isolated descriptions of such after-effects.

Own Observations.

As the combined sulphonamide-serum treatment led to a decided improvement of the prognosis for Pfeiffer meningitis in Denmark also (Brochner-Mortensen, Engbæk & Schmith, 1947) (7), it was not considered advisable, having regard to the former very grave prognosis and the relatively small number of patients, to abandon this therapy for treatment with streptomycin alone, experience of this being only small as yet, judging by the literature.

Now that streptomycin has become available in Denmark, it has been recommended that for the present all patients should as far as possible be treated with streptomycin as well as sulphonamide and type-specific rabbit immune serum.

Up to May 1st, 1947, this combined treatment was given to a total of seven patients from all of whom Pfeiffer's bacillus type b was cultivated from the spinal fluid.

The principles governing the serum and sulphonamide treatment are the same as those described in the above-mentioned work.

The child soon recovered, and the day after injecting the serum the spinal fluid was sterile. Urticarial exanthema seen on 10th day, but without much tp. reaction.

Discharged quite cured on 29th day.

Summary.

As a result of treatment with type-specific antibody the lethality of patients suffering from Pfeiffer meningitis was reduced considerably.

At the Blegdam Hospital seven patients were treated; two died.

Bibliography.

1. Aleman, R.: New Orleans M. & S. J. 93: 25, 1940. — 2. Alexander, H. E.: Proc. Soc. exp. biol. Med. 40: 313, 1939. — 3. Alexander, H. E.: Bull. New York Acad. Med. 17: 100, 1941. — 4. Alexander, H. E.: Am. J. Dis. Child 66: 172, 1943. — 5. Alexander, H. E.: J. Ped. 25: 517, 1944. — 6. Alexander, H. E.: J. Ped. 29: 192, 1946. — 7. Alexander, H. E., E. Ellis & G. Leidy: J. Ped. 20: 673, 1942. — 8. Alexander, H. E. & G. Leidy: Science 104: 101, 1946. — 9. Alexander, H. E., G. Leidy, G. Rake & R. Donovick: J. A. M. A. 132: 434, 1946. — 10. Alexander, H. E. & M. Heidelberger: J. exp. Med. 71: 1, 1940. — 11. Alexander, H. E., M. Heidelberger & G. Leidy: Yale J. Biol. Med. 16: 425, 1944. — 12. Arnett, J. H., G. D. Shoup & N. W. Henry: Am. J. M. Sc. 200: 674, 1940. — 13. Birmingham, J. R., R. Kaye & M. H. D. Smith: J. Ped. 29: 1, 1946. — 14. Bjorneboe, M. & J. Clausen: Nord. Med. 1: 906, 1939. — 15. Boisvert, P. L., M. D. Fovsek & M. F. Grossman: J. A. M. A. 124: 220, 1944. — 16. Davies, J.: Lancet 244: 553, 1943. — 17. Engbæk, H. C., N. I. Nissen & A. H. Schleisner: Ugeskr. f. læger 103: 1599, 1627, 1941. — 18. Engbæk, H. C.: Ugeskr. f. læger 110: 145, 1948. — 19. Fothergill, L. D.: New England J. Med. 216: 587, 1937. — 20. Fraenkel, E.: Ztschr. f. Hyg. u. Infektionskr. 27: 314, 1898. — 21. Grelland, R.: Tidsskrift for den norske lægeforening 66: 463, 1946. — 22. Grob, W.: Arch. f. Kinderh. 124: 59, 1941. — 23. Jenks, H. R. & S. Radbill: Arch. Pediat. 48: 1, 1931. — 24. Knouf, E. G., W. J. Mitchell & P. M. Hamilton: J. A. M. A. 119: 687, 1942. — 25. Lamm, S. S. & B. H. Shulman: J. Pediat. 24: 408, 1944. — 26. Lindsay, J. W., E. C. Rice & M. A. Selinger: J. Pediat. 17: 220, 1940. — 27. Logan, G. B. & W. E. Herrell: Proc. Staff. Meet. Mayo Clinic 21: 393, 1946. — 28. Mac Pherson, C. F. C., M. Heidelberger, H. Alexander & G. Leidy: J. Immun. 52: 207, 1946. — 29. Neal, J. B.: New York State J. Med. 33: 94, 1933. — 30. Neal, J. B., E. Appelbaum & H. Jackson: J. A. M. A. 115: 2055, 1940. — 31. Nichols, D. R. & W. E. Herrell: J. A. M. A. 132: 200, 1946. — 32. Nussbaum, S., S. Goodman, C. Robinson & L. Ray: J. Ped. 29:

- 14, 1946. — 33. Palmer, J.: Staff. Meet. Honolulu Clin. *12*: 173, 1946. — 34. Pfeiffer, R.: Deutsche med. Wchnschr. *18*: 28, 1892. — 35. Pittman, M.: Proc. Soc. Exper. Biol. & Med. *27*: 299, 1930. — 36. Pittman, M.: J. exp. Med. *53*: 471, 1931. — 37. Pittman, M.: J. exp. Med. *58*: 683, 1933. — 38. Sako, W., C. A. Stewart & J. Fleet: J. Pediat. *25*: 114, 1944. — 39. Scully, J. P. & M. L. Menton: J. Pediat. *21*: 198, 1942. — 40. Silverhorne, N. & A. Brown: J. Pediat. *16*: 456, 1940. — 41. Slawyk: Ztschr. f. Hyg. u. Infektionskr. *32*: 443, 1899. — 42. Smith, M. H. D., P. E. Wilson & H. L. Hodes: J. A. M. A. *130*: 331, 1946. — 43. Weinstein, L.: New England J. Med. *235*: 101, 1946. — 44. Wilkes-Weiss, D. & R. W. Huntington: J. Pediat. *9*: 462, 1936.
-

From the State Serum Institute, Copenhagen.
(Director: J. Ørskov, M. D.).

Pfeiffer Meningitis.¹

Treated with Streptomycin, Sulphonamide and Type-Specific Rabbit Immune Serum.

By

HANS CHR. ENGBÆK.

(Submitted for publication September 4, 1947.)

Waksman et al. proved in 1944 that streptomycin has a bactericidal effect on Gram-negative rods. The effect on Pfeiffer's bacillus was later studied in vitro and in vivo by Alexander et al. (1946) (1, 2).

Fifty type b strains from patients not treated with streptomycin were all inhibited totally by concentrations of from 0.5 to 10.8 units/ml medium, which means that all these strains were very sensitive to this agent.

After coming under the influence of streptomycin it appears that the sensitivity of some strains is reduced considerably, so that they are not inhibited by concentrations of up to 1000 u/ml medium. In such cases, treatment with streptomycin is without effect. Alexander (1946) (1), Alexander et al. (1946) (3) Birmingham et al. (1946) (6) and Weinstein (1946) (12).

Alexander & Leidy (1947) (3) have shown that in bacteria cultures of ten different strains of Pfeiffer's bacillus there were in all instances small numbers of strongly resistant bacteria within an otherwise sensitive culture. The number of such bacteria in strains isolated prior to treatment is no greater for patients who react only poorly to streptomycin than for those who react promptly to the treatment. Consequently, it is to be assumed that the development of purely streptomycin-resistant strains depends more

¹ Carried out with support from P. Carl Petersen Foundation.

on the rate of multiplication than on the primary number of resistant bacteria in the culture. In this Alexander & Leidy find an explanation of the fact that slight and moderately severe cases (in which the rate of multiplication of the bacteria is probably lower) are very often cured with streptomycin alone (Alexander et al. (1946) (5), Alexander & Leidy (1947) (4), Weinstein (1946) (12), Birmingham et al. (1946) (6).

The acquired increase of resistance is constant through many passages, both in vitro and in vivo.

In the U.S.A. in 1946 were published a number of cases of Pfeiffer meningitis which were treated with streptomycin, either alone or combined with sulphonamides and type-specific rabbit immune serum. The streptomycin was injected both intramuscularly and intrathecally because of its slight diffusion from the blood to the spinal fluid.

As already stated, slight and moderately severe cases were cured in many instances by streptomycin treatment alone, but where severe acute and chronic cases are involved, streptomycin treatment alone is inadequate. Here it is advisable to administer type-specific rabbit immune serum and sulphonamide at the same time. Furthermore, this treatment is always indicated for children younger than seven months owing to the very grave prognosis in this age group (Alexander et al. (1946) (5), Nichols & Herrell (1946) (11), Logan & Herrell (1946) (10), Alexander & Leidy (1947) (4).

In 1946(9) the Committee on Chemotherapeutics and other Agents, National Research Council, published 100 cases of Pfeiffer meningitis treated with streptomycin. Of these patients, 17 died — a number which corresponds to the lethality in the combined serum and sulphonamide treatment. Of those cured, only 18 were treated with streptomycin alone — the others were also given penicillin, sulphonamide or immune serum in various combinations. The material shows that streptomycin is often least effective when given late in the course of the disease after other treatment has failed. Therefore, treatment with this agent should begin early after the onset.

In this material too there were a few streptomycin-resistant strains against which the treatment had no effect.

Treatment with streptomycin for Pfeiffer meningitis mostly being of short duration, no serious toxic symptoms from it have been observed.

Treatment with streptomycin is begun as soon as the bacteriological diagnosis is arrived at, and the penicillin treatment is discontinued simultaneously. Streptomycin is usually injected every three hours intramuscularly in doses of 100 mg and intrathecally once a day in doses of 25 to 50 mg. This treatment is continued for six to eight days.

Six of the seven patients were cured. The one who died (No. 14) was four months old. Streptomycin was given after about four weeks from the onset, the result apparently being a cure. Four weeks later, however, there was a violent relapse. The child was again treated with streptomycin and in addition rabbit immune serum corresponding to 100 mg specific nitrogen intraperitoneally; but the patient was already moribund when this treatment was begun. Post mortem examination revealed a firm, capsulated abscess in the left frontal region. This and another case (No. 9) will be published elsewhere.

The case records of the remaining five patients are given below. Temperature curves and details of laboratory tests and treatment are shown in figs. 1, 2, 3, 4, 5.

The six patients cured were 8, 11, 13, 14, 22 and 31 months old. The one who died was four months old.

Pfeiffer No. 10. ♂. A. 8 months. Hosp. 17-2-47, disch. 29-3-47. Cured. Previously well. For a month before admission ill with cough, nasal catarrh, bronchitis and temp. fluctuating from 38 to 40°. Treated four days with alphasol without effect. On admission to the Infant. Dept. on 17-2 pale, but not seriously ill. Meningeal symptoms found next day. The spinal fluid being turbid, treatment with sulphathiazol and penicillin commenced. Culture from the spinal fluid having given growth of Pfeiffer's bacillus type b, transferred to Blegdam Hosp. on 20-2. Patient very ill, screaming, with tense fontanelle, considerable strabismus, convulsions and pronounced rigidity of neck and back. Otological examination: natural. Sulphonamide treatment continued and rabbit immune serum corresponding to 100 mg specific nitrogen given intraperitoneally against Pfeiffer's bacillus type b. Next day worse, and spinal fluid not sterile. Excess of free antibody in serum found only after second serum injection. Simultaneously treated with streptomycin 100 mg intramuscularly every three hours and 100 mg intraspinally once a day. On 22-2 the streptomycin concentration in the blood three hours after the injection = 24 units per ml, on 27-2 an hour after the injection 58 units per ml. Decided improvement under this treatment and streptomycin discontinued after third day. The meningeal symptoms subsided and the child now quiet. As the temp. on 26-2 rose slightly and the spinal sugar fell to 29, the third serum injection given intraperitoneally to increase the antibody titre in the blood. The spinal fluid still being turbid and the patient again unwell, streptomycin resumed

as from 27-2. Patient quickly became better and now recovered completely. On 2-3 the meningeal symptoms disappeared. On 5-3 all treatment discontinued. The temp. still slightly high, however, during the next 17 days, presumably owing to the many infiltrations in nates after the injections. Slight serum exanthema 18 days after the first serum injection. Otherwise no discomfort from treatment. Discharged after being 41 days in hosp., quite well and with psychic development corresponding to age.

For details of laboratory findings and treatment see fig. 1.

Pfeiffer No. 11. ♂. W. A. 14 months. Hosp. 10-3-47, disch. 22-4, cured. Ailing the last four or five months before present affection. Paracentesis duplex about a month ago, followed by abundant discharge from both ears. Owing to temp. increase the child hosp. a few days later (14-2) in the Infant Dept. Treated with penicillin for 10 days, then sulphathiazol for three days. Ears still discharging and temp. higher (40.2°), therefore transferred to Rigshospital Otological Dept. Not seriously ill. No meningeal symptoms. Purulent secretion from both ears. Tympani swollen and red. Mastoid region left and right natural. After transient fall of temp. it rose again. On 13-3 the spinal fluid turbid, but no bacteria found by direct microscopy. After penicillin treatment begun, operation resectio proc. mastoid. d. et s. performed. The bone somewhat soft, the cells full of a rather slimy, ropy secretion. Cultivation from this gave no Pfeiffer's bacillus. No abscess or pus found. During following days treated with penicillin intramuscularly and sulphonamide by mouth. After the operation a temporary fall of temp. A culture from the spinal fluid on 13-3 having given Pfeiffer's bacillus, streptomycin begun on 14-3, 100 mg intramuscularly every three hours and 40 mg intrathecally once a day for 7 days. Treatment with sulphonamide by mouth continued during the first days. On 14-3 also given type-specific rabbit immune serum for Pfeiffer's bacillus type b intraperitoneally corresponding to 100 mg specific nitrogen. Five hours after the intrathecal inj. of streptomycin and four hours after the serum inj. the patient very limp, pale, poor pulse, but quickly recovered. The antibody titre in the blood having remained at 2-4, further serum treatment not indicated. Patient not seriously ill at any time and quickly got well under this combined treatment. On 14-3 and 15-3 the streptomycin concentration in the blood an hour after 100 mg intramuscularly: 64 and 48 units per ml. Temp. fell to normal, where it remained, apart from a brief rise eight days after treatment discontinued. This attack not due to a flaring up of the meningitis but probably a slight sore throat. Operation cavities healed after about three weeks. Except for the said reaction — which possibly due to the serum injection — and a temporary diffuse exanthema, no discomfort from the treatment observed. Patient discharged cured after 70 days in hospital.

For details of laboratory findings and treatment see fig. 2.

Pfeiffer No. 12. ♀. B. H. 13 months. Hosp. 24-3-47, disch. 26-4-47. Cured. Formerly well. Ill ten days before hosp. with transient temp.

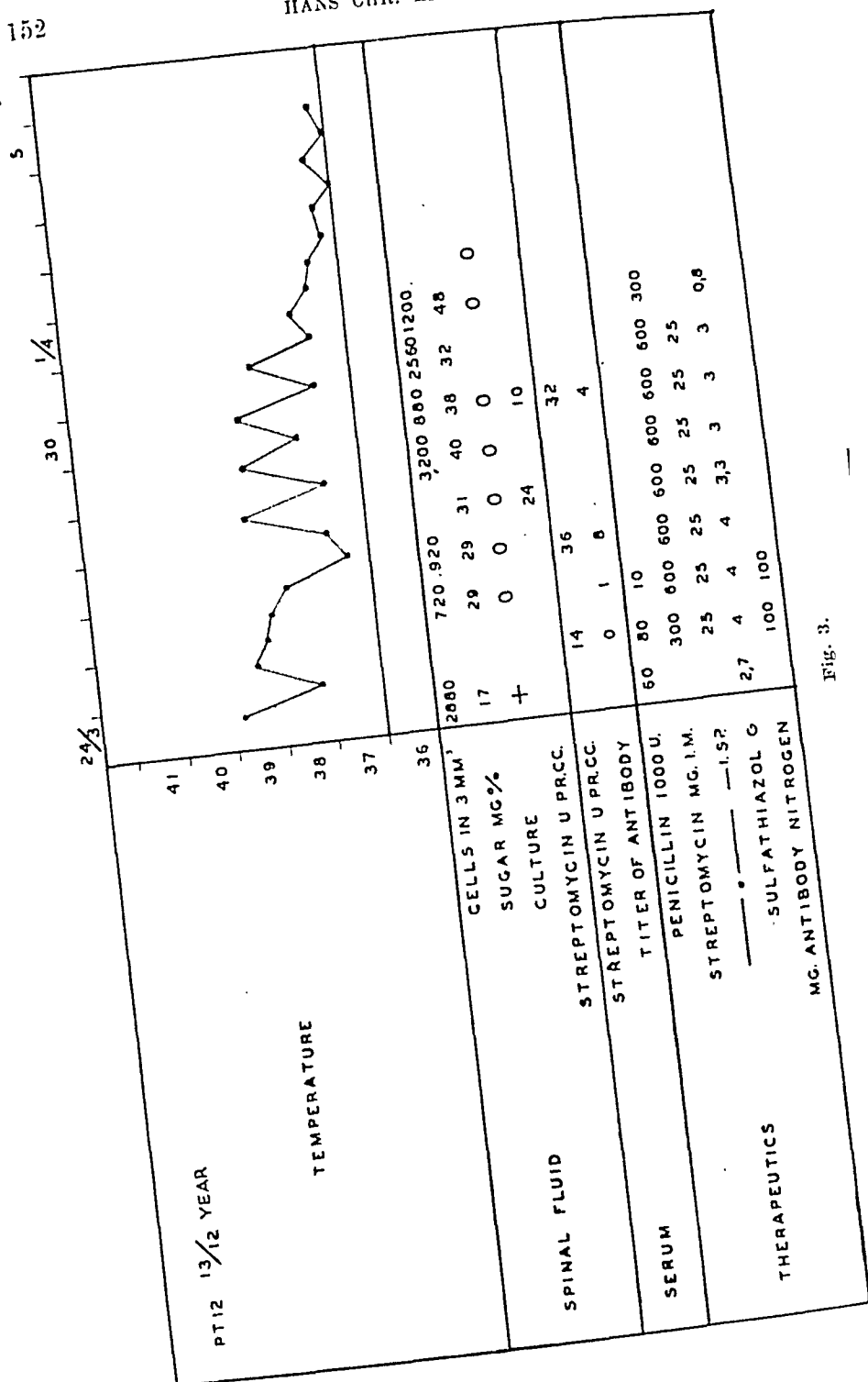


Fig. 3.

and malaise. No meningeal symptoms. Last four days increasing malaise, restless and screaming. Day before adm. incipient meningeal symptoms and slight squint in right eye. On adm. to Central Hosp., Slagelse, very ill with strong meningeal symptoms and paresis of right n. abducens. Spinal fluid slightly turbid and containing Gram-negative rods which on cultivation proved to be Pfeiffer's bacillus type b.

Treatment commenced at once with intramuscular inj. of penicillin and sulphonamide by mouth. Immediately after the bacteriological diagnosis, treatment instituted with streptomycin intramuscularly every four hours and intrathecally every day for seven days. Sulphonamide treatment continued. On 25-3 and 26-3 also given type b/rabbit immune serum corresp. to 100 mg specific nitrogen intraperitoneally. On 25-3 the streptomycin content in the blood two hours after the first streptomycin inj. = 36 units per ml, on 30-3 an hour after inj. = 32 units per ml. General condition improved fairly quickly and temp. fell lytically. Meningeal symptoms subsided and had disappeared by the 30-3. On 2-4 the squint had gone. Disch. cured after 33 days, completely well. Apart from a slight, universal urticarial exanthema on 29-3 and 30-3, no discomfort from treatment.

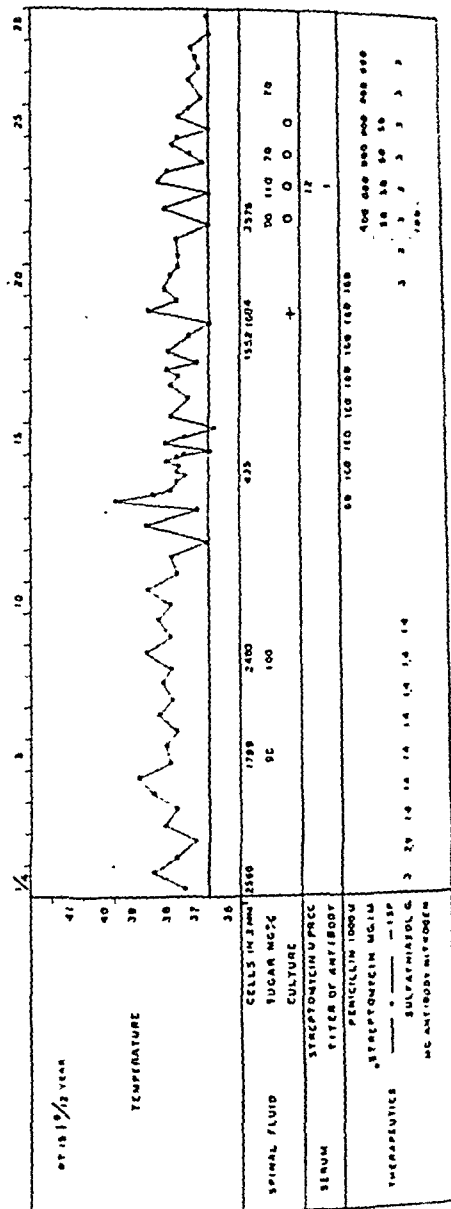
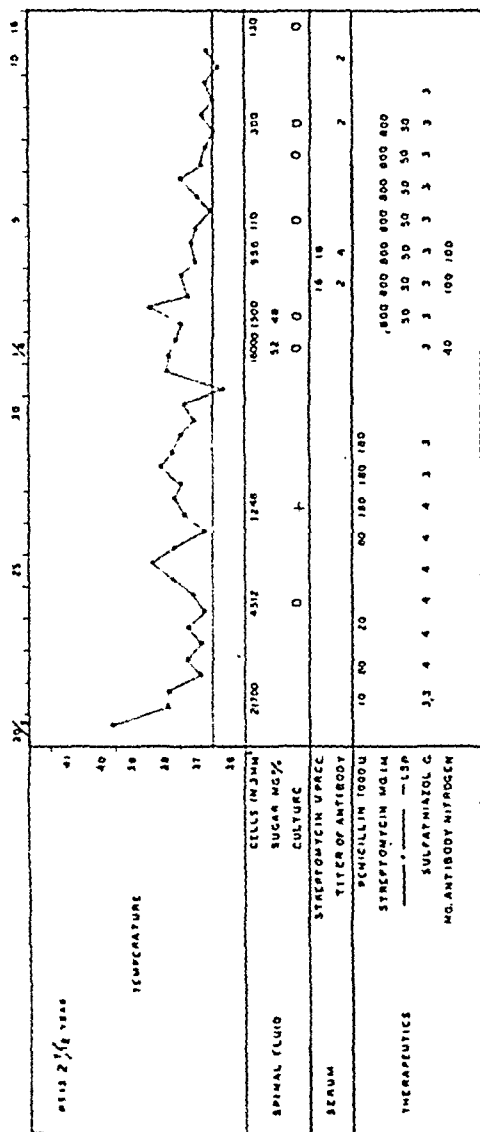
For details of laboratory findings and treatment see fig. 3.

Pfeiffer No. 13. ♀. N. D. 31 months. Hosp. 21-3-47, disch. 21-4-1947. Cured. Previously well. Acutely ill 36 hours before admission to Horsens Munic. Hosp. with temp. 40°, vomiting, convulsions and strabismus. No cough, no catarrh.

Very ill, wandering, slight neck and back rigidity. Otoscopy natural. Spinal fluid sterile, but no bacteriological diagnosis made by cultivation, wherefore treated with penicillin intramusc. every three hours and sulphonamide by mouth. Result was a sudden fall of temp., but not to the normal. General condition considerably improved. Patient quite conscious, moved freely but still slightly meningeal. Despite penicillin and sulphonamide treatment for seven days, Pfeiffer's bacillus type b cultivated from spinal fluid on 27-3. This diagnosis not being made until later (1-4-47), treatment was discontinued on 30-3; two days later condition exacerbated acutely, meningeal symptoms increased, temp. rose and spinal fluid more turbid. Although no growth of Pfeiffer's bacillus from this, it was undoubtedly a flare-up of the meningitis. When the bacteriological diagnosis on the spinal fluid was available treatment commenced on the 14th day of the disease with streptomycin 100 mg intramusc. every three hours and 50 mg intrathec. once a day for eight days. Simultaneously sulphonamide given by mouth. On 3-4 and 4-4 given rabbit immune serum intraperit. against Pfeiffer's bacillus type b corresponding to 100 mg specific nitrogen. A small dose (40 mg) found in stock at the hosp. was given on 1-4.

General condition improved quickly and symptoms subsided. No discomfort observed from treatment. Streptomycin concentration in blood three hours after 100 mg intramusc. on 3-4 and 4-4 = 16 and 18 units per ml.

Patient discharged completely cured after 32 days in hosp. For details of laboratory findings and treatment see fig. 4.



Pfeiffer No. 15. ♂. D. K. 21 months. Hosp. 1-4-47, disch. 3-5-47. Cured. Previously well. Last five days before hosp. drowsy, whimpering. High temp. observed only day before hosp. (38.4°). Not seriously ill on adm. to Hospital, Nykøbing M., but malaise with slight meningeal symptoms. No catarrh or cough. Otoscopy natural. Spinal fluid purulent, but no bacteriological diagnosis. Treatment with sulphonamide commenced immediately and continued 9 days. After three days' pause treated with penicillin intramusc. every three hours for eight days. In this period the general condition practically unchanged and temp. still high. On 19-4 sub-occipital puncture, the fluid cultured and proved to contain Pfeiffer's bacillus type b, wherefore as from 22-4, on the 27th day of the disease, treated for six days with streptomycin 100 mg intramusc. eight times a day, and for four days also 50 mg intrathecally once a day. On 22-4 given rabbit immune serum intravenously for Pfeiffer's bacillus type b corresponding to 100 mg specific nitrogen. Despite negative conjunctival test with normal rabbit serum prior to injection there was a fairly strong allergic reaction to the latter inj. with coughing, change of colour and exanthema, though recovery was quick after 1 ml. adrenalin. In the same period sulphonamide was also given by mouth. During the treatment the temp. fell lytically to normal, spinal fluid became sterile and general condition improved. Streptomycin concentration 12 units on 23-4, 45 minutes after intramuscular injection of 100 mg. Discharged completely cured after 33 days in hosp.

For details of laboratory findings and treatment see fig. 5.

Serum treatment. One of the seven patients (No. 15) had a strong allergic reaction during the serum injection (despite negative conjunctival test with normal rabbit serum), wherefore serum was discontinued, although the antibody titre after 24 hours was only 1.

Another patient (No. 11), had a rather severe collapse five hours after an intrathecal streptomycin injection and four hours after the serum injection, but the child soon rallied. As the antibody titre in the blood remained at 2—4, further serum treatment was not indicated in this case.

However, patients No. 10—12 and 15 showed that a single serum injection corresponding to 100 mg antibody-N is not always sufficient for producing such an excess; but it was secured by the second injection 24 hours later.

Patient No. 10, furthermore, makes it clear that, as has already been pointed out, there is a risk of serum underdosing if one employs Alexander's dosage table on the basis of the spinal sugar concentration.

In no case did the streptomycin treatment produce toxic symptoms. It was injected intramuscularly in the six cases every

three hours, in one case (No. 12) every four hours. All were also given one dose in 24 hours intrathecally.

The spinal fluid quickly became sterile with this combined treatment and remained sterile in all the patients who recovered; at the same time the general condition improved rapidly.

With regard to patient No. 10, who was in a grave condition, the first serum injection was given 24 hours before the commencement of the streptomycin treatment. Improvement was observed only after the second serum injection — which produced an antibody excess in the blood — and streptomycin both intramuscularly and intrathecally. In the other cases serum and streptomycin were commenced together, and therefore it is impossible to differentiate between the rôles played by the different therapeutics in the rapid improvement and complete recovery.

While the treatment was going on a number of tests were made for the streptomycin concentration in blood and spinal fluid.¹ The tests were few, but they show that after an intramuscular injection of 100 mg streptomycin the lowest concentration in the blood in this material from 45 mins. to 3 hrs. after the injection was 12 units per ml serum.

The Pfeiffer bacilli isolated from the seven patients were all inhibited by streptomycin in concentrations of ≥ 3 units per ml fluid medium.

One point of special interest is that the strain isolated at the relapse of the patient who died (No. 14) was no more resistant to streptomycin than the other strains tested.

The blood concentration tests show that single doses of 100 mg intramuscularly every three hours will probably maintain concentrations that are inhibitory to Pfeiffer's bacillus in children of up to two or three years. Similar tests of spinal fluids show that 24 hours after intrathecal injection of from 25 to 100 mg there are still concentrations of streptomycin capable of inhibiting Pfeiffer's bacillus.

Sulphathiazol is inhibitive to five of the strains tested in concentrations of ≥ 5 mg%. Strain No. 15 was inhibited by 10 mg%, but No. 14 required > 20 mg% to inhibit growth completely.

As in type-specific rabbit immune serum and streptomycin we have two effective therapeutics against purulent meningitis caused by Pfeiffer's bacillus, it is of paramount importance to make the

¹ These tests were made at the Institute of General Pathology, to whose chief, Professor K. A. Jensen M. D., I extend my best thanks.

bacteriological diagnosis without loss of time. As it is often impossible to find this bacillus by direct microscopy of the spinal fluid, and the type diagnosis in all cases can be made only by the capsular-swelling reaction, the spinal fluid should always be examined immediately at a special bacteriological laboratory.

The prognosis for Pfeiffer-meningitis being particularly grave for children under 2 years of age (in spite of sulphonamide treatment) (Engbæk, 1947), (8) all patients in this age group for the present should be treated immediately with streptomycin, sulphonamide and type-specific rabbit immune serum. The streptomycin should be injected by both the intramuscular and intrathecal routes. According to American experience, treatment for four or five days is adequate. As previously recommended, type-specific rabbit immune serum should be given in two injections, each of 100 mg specific antibody-N, at an interval of 24 hours. Six to twelve hours after the latter injection the antibody titre should be determined in the blood and the continued serum treatment dosed accordingly. The resistance of the bacterium to streptomycin should always be followed during the treatment.

Patients older than two years may, according to circumstances, first be treated with sulphonamide and serum early after the onset. If the reaction to this treatment is not favourable (better general condition, sterile spinal fluid with falling cell count and rising spinal sugar) in the course of 48 hours, they should also be given streptomycin. This combined treatment should always be applied immediately in case of a relapse.

Summary.

Six patients with Pfeiffer-meningitis were treated with streptomycin, sulphonamide and type-specific rabbit-immune serum. All recovered.

One patient (four months old) treated with streptomycin and sulphonamide improved temporarily, but had a relapse four weeks later due to a capsulated abscess and died, in spite of renewed streptomycin treatment. One serum injection a few hours prior to death did not affect the course of the disease.

All the strains of Pfeiffer's bacillus tested were sensitive to streptomycin.

It is thus advisable to treat immediately all children under

two years with streptomycin and sulphonamide as well as type-specific rabbit immune serum.

Children over two years may, according to circumstances, be treated first with sulphonamide and serum early after the onset. If the reaction to this treatment is not favourable within 48 hours, they should also be treated with streptomycin. All three therapeutics should be applied immediately in case of a relapse.

References.

1. Alexander, H. E.: *J. Pediat.* 29: 192, 1946. — 2. Alexander, H. E. & G. Leidy: *Science* 104: 101, 1946. — 3. Alexander, H. E. & G. Leidy: *J. Exper. Med.* 85: 329, 1947. — 4. Alexander, H. E. & G. Leidy: *Am. J. Med.* II: 457, 1947. — 5. Alexander, H. E., G. Leidy, G. Rake & R. Donovan: *J.A.M.A.* 132: 434, 1946. — 6. Birmingham, J. R., R. Kaye & M. H. D. Smith: *J. Pediat.* 29: 1, 1946. — 7. Brøchner-Mortensen, K., H. C. Engbæk & K. Schmith: *Ugesk. f. læger* 110: 139, 1948. — 8. Engbæk, H. C.: *Ugesk. f. læger* 110: 145, 1948. — 9. Keefer, C. S. et al.: *J.A.M.A.* 132: 4, 1946. — 10. Logan, G. B. & W. E. Herrell: *Proc. Staff. Meet. Mayo Clinic* 21: 393, 1946. — 11. Nichols, D. R. & W. E. Herrell: *J.A.M.A.* 132: 200, 1946. — 12. Weinstein, L.: *New England J. Med.* 235: 101, 1946.
-

From the Hospital St. Antoniushove, Voorburg, Holland.
(Dr. Th. F. Bloem, Medical Director.)

Sulphasuccidine a Cause of Polyneuritis.

By

G. A. GUSSENHOVEN.

(Submitted for publication October 6, 1947.)

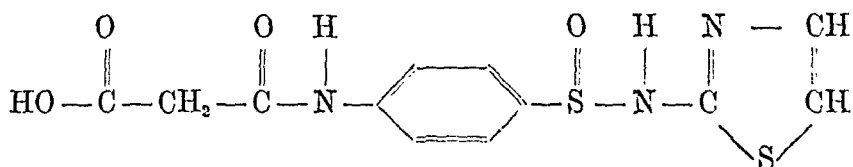
Sulphasuccidine was introduced into the clinic by Poth c. s. (1—3) in 1941 and given ante and post operationem to prevent or cure inflammations of the colon and rectum and as treatment for bacillary dysentery and chronic colitis.

Sulphasuccidine is $C_{13}H_{13}N_3O_6S_2H_2O$:

2-(N₄-succinylsulphanilamido)thiazole monohydrate or

2-(p-succinylaminobenzene sulphonamido)thiazole monohydrate.

Its molecular weight is 373.4 and the structure formula is:



It is a crystalline white powder without a definite smell and by body temperature 70 mg is dissolved in 100 ml water. Contrary to the sulphaguanidine, which is used more frequently and of which up to 50 % is excreted in the urine (4), it was stated that only 5 % of the totally administered amount of the sulphasuccidine was excreted through the urine (5). It was supposed that the sulphasuccidine was hydrolyzed in the intestines and so sulphathiazole thus formed, handicaped the growth of harmful elements of the intestinal flora. The poor resorption was supposed to be the cause of the rare occurrence of harmful consequences on administering the drug. Clay and Pickrell however saw a case of

crystal formation in the kidneys with haematury (6) and Johnson mentions a case of acute agranulocytosis (7). Affections of the peripheral nervous system by sulphasuccidine are as far as we know not mentioned in the literature.

Report of Case.

History. Mrs. C. v. d. S.-P., a married woman, 36 years old, was admitted to the hospital on 15/1 1946. Already 2 years she suffered from diarrhoea of varying intensity. During the period of starvation (1944—1945) her stools were mixed with blood and mucous. She had a temperature and had to stay in bed for two months. Two months before admission she began to complain of cramps in the left part of the lower abdomen, especially before and after defecation. The appetite was good. As far as possible she had tried to live on a diet poor in cellulose. The serious scantiness of food during the last year of the war made her diet very insufficient in quality and quantity.

Past Medical History: In 1937 the patient was operated on for a retroflexio uteri; in 1942 abdominal adhesions were removed.

Physical examination: The patient was well developed and nourished. The blood pressure was 110/85; pulse, temperature and respiration were normal. There was no anaemia, the skin turgor was good. The tongue was slightly atrophic. Heart and lungs were essentially normal. There was no visible peristalsis; the lower abdomen, symmetrically swollen, showed a horizontal operation-scar. The uterus was that of a 5 months pregnancy. The abdomen was diffusely painful on pressure; pressure on the lower left quadrant was particularly painful. The colon was not palpable, nor the liver, the spleen or the kidneys. Percussion and auscultation of the abdomen were normal. The patient had large external haemorrhoids.

Laboratory Data: Urinalysis of a catheterised specimen was negative. Blood analysis stated 8,250 leucocytes and 3,620,000 erythrocytes with 70 % haemoglobine. The sedimentation rate was 27 mm (after 1 hour) and the haemogram showed 10 rod cells without further abnormalities. The serological reactions of Wassermann, Meinicke and Müller were negative and the ureum level of the blood was 30 mg%. There was neither sanguis in the faeces, nor digestive faults or worm eggs. Repeated agglutination tests for dysentery were negative. Fractional examination of the stomach contents showed a histamine resistant achylia gastrica. Spectroscopic examination of the stomach contents was negative.

Roentgenologic Observation: 8 hours after the oral administration of barium the whole of the colon was filled and haustrations were regular. On account of the gravidity there was only a minimum of X-ray scopy and no photographs were made.

Therapy: Diet poor in cellulose, pepsin-hydrochloric acid with meals and sulphasuccidine per os, 6 grams daily.

Course: After two weeks her defecation was less frequent. Four times a day she produces a mucous, pulpy bowel without blood. The content of sulphasuccidine in the blood was 2.3 mg%. On February the 9th the abdomen was less painful on pressure but her defecation showed no further improvement. Sulphasuccidine level of the blood was 1.9 mg%. A total amount of about 150 gram sulphasuccidine was given and besides that the next two weeks three times a day 500 mg dermatol.

On February the 24th there was still no improvement worth mentioning: instead of dermatol, beer-yeast was added three times a day two tea-spoonfuls.

On March the 9th, four weeks after the stopping of the sulphasuccidine, the patient complained of violent paraesthesias, continual tickling and tingling in all extremities. There was fierce pain by pressing the peripheral spinal nerves and distinct hypaesthesia for all qualities in the extremities. The consulting neurologist confirmed the diagnosis polyneuritis and advised intravenous administration of 100 mg thiamine twice a week.

On April the 8th the patient still complained of violent pain in the extremities. The tendon reflexes had disappeared, all movements were paretic, especially of the legs, the patient was unable to stand upright. The muscles of the extremities were atrophic and showed a degeneration reaction. There were no disturbances of the sensorium and no anomalies of the papillae nervi optici. In view of the gravity a lumbar puncture was not done.

After the partus à terme on May the 2nd the examination could be extended. Rectoscopic abnormalities were not found. The roentgenologic examination was repeated and a barium-enema was given. The barium entered easily, without passing the valvula Bauhini. The haustration of the colon ascendens was scanty, afterwards ragged, especially in the aboral part. The colon was very long and winding. The X-photographs, made after defecation showed the caudal colon and the sigmoid without haustration and this part did not reveal a sign of mucosa configuration, giving right to the conclusion that the inflammation of the colon was localised in the caudal part. To complete the neurologic examination a lumbar puncture was made. The liquor proved to be normal. On September the 14th the patient showed symptoms of a right-sided lung emboly, the origin of which is not quite evident, but might be explained as a consequence of the delivery 4 months previous.

Slowly the patient recovered of her polyneuritis. Not before seven months after the first appearance of the polyneuritis the reflexes were normal and the muscles reacted normally. The strength increased and by means of massage the patient could be mobilised on November the 12th. She was discharged on November the 22nd, ther strength being normal. There were only slight superficial disturbances of the sensibility. The stools have remained pulpy, without blood or mucous, the defecation occurred nearly three times a day without pain. She continued her diet poor in cellulose, pepsinhydrochloric acid and thiamine per os.

Polyneuritis in consequence of the use of sulphanilamide preparations occurs only once or twice in 1,000 cases, according to Frisk, who has made an extensive literary study on this subject (8).

The sulpha-drugs with a methyl group are in general the most harmful to the peripheral nervous system.

The sulphasuccidine is probably less poisonous on account of its poor resorption in the intestines. We assumed that the described patient had a polyneuritis by several factors, but considered the sulphasuccidine as *conditio sine qua non* of her affection, because the other factors had asserted their influence already a long time before the sulphasuccidine was given. These predisposing factors are:

- I. The achylia gastrica; it is known that there are disturbances of the nervous system analogic to those of the morbus Addison-Biermer, without blood changes (9).
- II. The thiamine stock of this patient certainly must have been bad.
 - a. her nourishing was, as of all the Dutch people, very bad during the past war.
 - b. the diarrhoea has had a bad influence of the resorption of the thiamine. Polyneuritides in the course of chronic enterocolitides are described (10).
 - c. during the gravidity there is an increased use of thiamine (11). The older literature mentions several cases of polyneuritis gravidarum.
 - d. the sulphasuccidine decreases the level of thiamine in the blood (12).

It is unlikely that the patient was hypersensitive for sulphasuccidine. Common symptoms of allergy as exanthems, drug fever and eosinophilia were never found.

The latent period between taking the medicine and the first appearance of the polyneuritis concurs with that of the polyneuritis in consequence of »Jamaica Gin» and the injury of the nervous system by triorthocresylphosphor acid, which it contains.

Summary.

Description is given of a woman, aged 36, 5 months pregnant, who suffered from colitis during two years. A month after the administration of about 150 gram sulphasuccidine, divided over 25 days, she had a serious polyneuritis. The sulphasuccidine must

be considered as *conditio sine qua non* of this polyneuritis, the achylia gastrica and the B₁₂-hypovitaminosis may have served as predisposing factors.

References.

- 1) Poth and Knott: *Proc. Soc. Exp. Biol. and Med.* 48, 129, 1941.
- 2) Poth and Knott: *Arch. Surg.* 44, 208, 1942. — 3) Poth and Knott: *J. Lab. Clin. Med.* 48, 129, 1942. — 4) Frisk: *Acta Med. Scand. Suppl. CXLII*, 102, 1943. — 5) Poth: *J. A. M. A.* 120, 265, 1942. — 6) Clay and Pickrell: *J. A. M. A.* 123, 203, 1943. — 7) Johnson: *J. A. M. A.* 122, 668, 1943. — 8) Müller: *Acta Med. Scand. CXXI*, 108, 1945. — 9) Gans: *Leerboek der Neurologie*, pag. 469. — 10) Wilke, *Deutsche Med. Wochenschrift* 23/24, 443, 1943. — 11) *Nutrition Reviews* 3, 248, 1945. — 12) Najjar and Holt: *J. A. M. A.* 123, 683, 1943.

Institut d'histologie spéciale de l'université de Gand (Belgique).
(Dir.: Prof. De Groodt A.)

L'endarterite oblitérante et l'endophlébite en cas d'ulcère de l'estomac.

Par

GEORGES De BUSSCHER.

(Bruges, Belgique.)

(Ce travail est parvenu à la rédaction le 26 Septembre 1947.)

Examen macroscopique:

Si nous injectons une substance contrastante (lipiodol, iodopine, néodipine, encre de Chine blanche, solution gélatineuse au baryum) dans une ou plusieurs des artères d'un estomac réséqué, nous remarquons sur le cliché radiographique, que le processus d'endarterite atteint brusquement l'artère c.à.d. que très rapidement la lumière des artères périulcéreuses diminue de calibre, s'effilant, formant un filet mince ou subissant un arrêt complet: c'est l'expression radiologique du processus d'endarterite oblitérante observé en cas d'ulcère du ventricule. Ce phénomène se rencontre aussi loin que s'étend l'anneau périulcéreux d'induration; il est proportionnel à son intensité et s'arrête brusquement à ses bords (il existe même une vascularisation accrue autour de la zone d'induration périulcéreuse et une adaptation du calibre des autres artères gastriques).

Examen microscopique; revue de la littérature.

L'endarterite oblitérante peut avoir comme point de départ différentes couches de la paroi artérielle, son mécanisme de production est complexe.

L'endothèle peut être le point de départ d'une prolifération ou d'un bourgeonnement proéminent dans la lumière vasculaire à base sessile ou pédiculée. (Winniwarter, Masson, Mc Kelvey et Mc Mahon, Lindenberg et Spatz.)

Il peut se produire une déhiscence de l'endothèle avec exsudation de cellules et de sucs qui s'organisent, l'endothèle les recouvre secondairement (Rössle—Baner, Coronini—Oberson).

Cette formation de bourgeons fibrinoides endartériels a été décrite de même par Wiese et Jäger.

L'endothèle reste pourtant le plus souvent typique.

La couche sousendothéliale peut être le siège d'une prolifération ou d'une production cellulaire conjonctive (Chiari, Jores, Friedländer, Siegmund, Papp) ou musculaire (Westphalen, Szas-Schwarz, Von Pakow et Sohna, Masugi-Ya-Shu), parfois en rapport avec l'adventice à travers la média (Masugi-Ya-Shu). Ceci pose tout le problème du passage de cellules conjonctives ou mésenchymateuses à travers l'élastique interne et à travers la média.

Le rôle joué par la substance fondamentale mésenchymateuse, que l'on retrouve le plus facilement dans la paroi des artères de calibre moyen ou petit semble d'une importance primordiale dans l'épaississement de la paroi, dans la formation des couches élastiques stratifiées et du tissu conjonctif. Ce tissu mésenchymateux peut acquérir des propriétés embryonnaires de sorte qu'il devient décelable, d'une manière plus ou moins parfaite, par nos méthodes histologiques de coloration.

Le développement postembryonnaire forme de l'intima une membrane serrée en rapport avec l'élastique interne (Westphalen, Jores, Torhorst, Voigts): sa lamelle interne s'épaissit par la formation d'une ligne homogène qui présente peu d'affinité pour les colorants de l'élastine; par après, les lamelles s'écartent partiellement ou entièrement de l'élastique interne par la formation d'une substance interstitielle; ce processus continue jusqu'à ce qu'il se soit formé un réticulum de fibres élastiques.

D'après Hueck, quand l'intima prolifère, il se produit un accroissement de la substance fondamentale conjonctive; par imprégnation, il se forme une nouvelle membrane élastique plus centrale; la substance fondamentale forme une couche intermédiaire spongieuse à travers laquelle les noyaux de la média se glissent, ils ont un aspect mésenchymateux.

Le rôle joué par la média ou le sort de cette couche n'a été considéré que par peu d'auteurs comme d'une importance primordiale dans la production de l'endartérite: accroissement (Sternberg); atrophie (Papp); nécrose (Bredt); destruction de la paroi élastico-conjonctive et musculaire de la média (Coronini—Oberson).

Hansbrandt a décrit les modifications de l'endothèle et du tissu sousendothélial (activation embryonnaire des cellules mésenchymateuses) dans la tuberculose expérimentale du cobaye. La paroi du vaisseau peut finalement devenir hyaline et la lumière s'oblitérer.

Comme facteurs étiologiques de ces modifications de structure on a considéré que le processus était d'origine inflammatoire, d'origine mécanique par variation de la pression sanguine (Bencke et Pekelharing) ou par ralentissement de la circulation (Thoma), d'origine allergique hyperergique (Rintelen).

Étude personnelle:

Le matériel se compose de quelques ulcères du bulbe, d'une série d'ulcères du ventricule gastrique, d'un ulcère double du pylore et du ventricule, présentant une forte formation de tissu conjonctif.

Fixation formol 10 % ou Bouin; coloration à l'hématoxyline-éosine, Weigert-résorcine, trichrome Masson et trioxyhématine de fer suivant Hansen.

A. Ulcère de bulbe; avec peu de tissu conjonctif de néoformation.

Si on examine les capillaires et les artérioles à partir du fond de l'ulcère vers la séreuse, on constate que ces capillaires et ces artérioles présentent le plus souvent une structure normale bien que certains d'entre eux apparaissent comme turgescents, c.à.d. que les cellules sont arrondies, que le noyau est rond et le protoplasme clair. Les grandes artérioles ont une structure normale, certaines présentent un endothèle normal, mais dont les noyaux ne sont pas étirés ou allongés: ils sont proéminants dans la lumière. Entre cet endothèle et la média, se trouve une couche intermédiaire à éléments désordonnés et dont les cellules sont entourées d'un réseau de fibres élastiques; elle présente une fine couche de tissu conjonctif collagène.

Certains vaisseaux montrent en dedans de la média, une rangée de cellules rondes, turgescentes à noyaux sphéroïdes qui semblent proéminer dans la lumière; elles ont un protoplasme clair et afibrillaire, elles ne sont pas entourées de fibres élastiques du côté externe de sorte qu'elles semblent appartenir à la média. Tous les stades intermédiaires peuvent étre retrouvés comme s'il se faisait un encerclement progressif par des fibres élastiques d'une ou de plu-

sieurs couches cellulaires. Ces images ressemblent très fortement à celles décrites par Merckel.

Les grandes artères, situées en profondeur, présentent un endothèle aplati avec une couche sousjacent de tissu conjonctif riche en cellules qui sont entourées par des membranes élastiques. Ces cellules ont une direction différente des cellules des couches plus externes vis-à-vis desquelles elles semblent perpendiculaires ou obliques.

Dans la *sousséreuse* où l'on trouve assez bien ou beaucoup de tissu conjonctif de *néoformation*, les capillaires présentent un endothèle turgescent. Il en est de même pour les petites artérioles qui montrent une fine couche sousendothéliale, les noyaux de la couche musculaire sont également turgescents. Le tissu conjonctif entre les cellules musculaires a encore fortement augmenté dans les grands vaisseaux. Ces cellules musculaires encerclées ont des noyaux sphéroïdes, à disposition irrégulière par l'accroissement du tissu conjonctif collagène; leur cytoplasme est clair. La coloration de l'élastine démontre que ces cellules sont entourées de fibres élastiques bien que quelques unes ne présentent de l'élastine que du côté central.

Conclusion: en cas d'ulcère du bulbe avec peu de néoformation de tissu conjonctif;

1) les capillaires présentent un endothèle turgescent; d'ailleurs peu accusé s'il y a peu de tissu conjonctif de néoformation mais bien de la gastrite.

2) les artères moyennes présentent une couche conjonctive sousendothéliale avec des cellules désordonnées, délimitées par de l'élastine du côté central; d'autres vaisseaux artériels montrent plusieurs couches de cellules et de noyaux désordonnés entourées d'élastine. (Photo 5.)

3) les grandes artères présentent une plus forte néoformation de tissu conjonctif, une forte formation de couches élastiques stratifiées et une transformation progressive *centripète* des couches cellulaires pseudo-proliférantes.

B. Ulcère du ventricule.

1) *En dehors de l'ulcère, ou l'on ne rencontre pas de tissu conjonctif de néoformation, les artères ont un aspect normal.* (Photos 1 et 2.)

Dès qu'on se déplace vers la zone périulcéreuse macroscopiquement caractérisée, au point de vue artériel, par un rétrécissement

de la lumière artérielle après injection d'une substance contrastante (examen radiologique) et microscopiquement par une augmentation du tissu conjonctif dans toutes les couches de la paroi gastrique, on constate également un accroissement du tissu conjonctif collagène et élastique dans la paroi de l'artère. Cette transition apparaît brusquement à la limite du bourrelet d'induration périulcéreuse.

2) *Le fond de l'ulcère calleux:*

Nous entendons par «Fond de l'ulcère» non seulement la notion macroscopique c.à.d. le fond du cratère ulcéreux visible à l'œil nu mais aussi, notion histologique, toutes les couches sousjacentes, la séreuse comprise.

Les artérioles petites et moyennes: Immédiatement en dessous de l'exsudat fibrino-leucocytaire, dans le tissu conjonctif de néoformation, les capillaires et les petites artérioles montrent un endothèle turgescent; *les artérioles plus grandes* présentent, en dessous de l'endothèle, deux couches dont la plus périphérique est circulaire à noyaux fusiformes et à protoplasme fibrillaire; la couche, plus centrale, présente de gros noyaux épithéloïdes désordonnés avec une distribution inégale de la chromatine et un nucléole; le cytoplasme en est afibrillaire, vacuolaire. Le tissu conjonctif intercellulaire est minime, il y a peu de réticuline. La couche interne montre des fibres élastiques. Dans la sousséreuse, on ne retrouve que des artères normales, s'il n'y a pas de tissu conjonctif de néoformation; sinon il existe de l'endartérite oblitérante (Photo 3).

C. L'examen du fond d'un *ulcère perforé dans le pancréas, mais avec seulement un début de formation de tissu conjonctif dans la sous-muqueuse*, n'a montré que des artères normales bien que les cellules de la média paraissaient un peu rondes et la média un peu épaissie.

D. *L'ulcère du canal pylorique et l'ulcère du ventricule au niveau de l'angle gastrique avec une forte formation de tissu conjonctif:* nous avons sectionné perpendiculairement à la petite courbure de façon à prélever une série continue de blocs de tissu à enrober afin de pouvoir suivre au microscope le processus endartéritique à ses différents stades.

a) *à quelques centimètres de l'ulcère:* on ne trouve pas de modifications de la paroi de l'artère.

b) *plus près de l'ulcère:* les petits vaisseaux de la sousséreuse montrent, en dessous de l'endothèle, deux couches: une couche

centrale à noyaux ronds ou sphéroïdes, désordonnés, plus ou moins enrobés dans le tissu conjonctif; une couche plus périphérique de cellules musculaires circulaires à noyaux fusiformes; le tout est entouré par l'adventice.

Les artères plus grandes présentent plus de tissu conjonctif de néoformation; une élastique interne n'existe pas mais la coloration à la Weigert-résorcine montre un réseau de fibres élastiques dans le tissu conjonctif. (Photo 6.)

Il semble que le tissu conjonctif entre les cellules musculaires de la média augmente de sorte que les cellules musculaires typiques de la média changent progressivement de direction; leurs noyaux allongés prennent une direction de plus en plus oblique et perpendiculaire vis-à-vis de l'axe de l'artère, ils s'arrondissent. Les couches cellulaires périphériques sont nettement fibrillaires, tandis que les cellules enrobées dans le tissu conjonctif néoformé perdent rapidement leur caractère fibrillaire, ils présentent un cytoplasme clair. *On peut suivre tous les stades de transformation des cellules musculaires typiques en cellules polymorphes de la couche proliférante bien qu'il n'y ait qu'une seule couche de transition des cellules de la média.* Le tissu conjonctif de la média authentique (c.à.d. à morphologie inchangée) est en continuité intime avec le tissu conjonctif de la couche proliférante.

La disposition capricieuse des cellules et des noyaux est due à la prolifération inégale du tissu conjonctif intercellulaire. Les fibres élastiques forment un réticulum dans le tissu conjonctif proliférant; ce réticulum est incomplet et certaines cellules apparaissent comme uniquement entourées d'élastine à leur face centrale. *Dans la même artère on peut souvent retrouver tous les stades d'encerclement des cellules de la média par le tissu élastique, processus d'intégration dans la soi-disant intima.* (Photo 4.)

Dans les artères de calibre moyen, la couche proliférante est parsemée de fibres élastiques, la couche la plus périphérique étant la plus épaisse: elle représente »l'élastique interne«. (Photo 6.)

c) *à proximité de l'ulcère:* on observe un accroissement remarquable de tissu conjonctif. Dans les petites artères de toutes les couches (sous-muqueuse, musculaire et sous-séreuse) il apparaît une forte néoformation de tissu conjonctif intercellulaire dans la média, de sorte que certaines cellules de la média semblent incrustées.

Les artérioles présentent un accroissement progressif de la substance intercellulaire conjonctive collagène d'autant plus pronon-

cée qu'on s'éloigne de la lumière, quelques couches de cellules de la média sont englobées, elles perdent progressivement leurs caractéristiques de «cellules de la média» pour acquérir celles de «cellules de l'intima». Il persiste une élastique interne tandis que les couches périphériques ne présentent que de fines lamelles fragmentées. *Il est impossible que ces débris d'élastine puissent représenter l'élastique originale mais l'élastine apparaît progressivement dans les couches de plus en plus excentriques: la média est, en quelque sorte, envahie et transformée.* (Photos 7 et 8.)

Les grosses artères montrent un fort accroissement de tissu conjonctif; dans la partie centrale, le tissu conjonctif prédomine, refoulant les cellules qui doivent adopter une disposition irrégulière. Une couche moyenne présente souvent des cellules à noyaux allongés et à direction circulaire, rappelant la disposition de la média, une troisième couche plus périphérique contient moins de tissu conjonctif mais des noyaux désordonnés; le tout est délimité par une élastique interne. La média, à structure typique, est mince; elle présente du tissu conjonctif intercellulaire modérément accru et en rapport intime avec le tissu conjonctif des autres couches. (Photo 11.)

d) *au niveau de l'ulcère:* la prolifération du tissu conjonctif dans toutes les couches est très prononcée.

Les petites artères, situées dans la profondeur du fond de l'ulcère au milieu de l'énorme formation de tissu conjonctif, ont un endothélium indemne, plusieurs assises cellulaires de la média gardent leur structure typique. Plus on se rapproche de la périphérie de l'artériole, plus on constate du tissu conjonctif collagène bien qu'il se trouve plus de substance fondamentale entre les cellules que la coloration au trichrome Masson n'en indique. Il y a peu de fibres élastiques entre les couches cellulaires internes tandis que les couches périphériques, d'autant plus qu'on se rapproche de la périphérie, présentent de fortes lamelles élastiques de façon à ce que toute la média est garnie, dans ces «espaces» intercellulaires de fortes fibres élastiques; l'adventice seule se trouve en dehors de la couche d'élastine la plus excentrique.

Il apparaît ainsi que, même quand la média a gardé une structure typique, qu'il y a un accroissement prononcé d'élastine et de tissu conjonctif collagène entre les couches de la média et cela d'autant plus qu'on se rapproche des couches périphériques de l'artériole. Les vaisseaux artériels ne gardent pas toujours au même degré et au même endroit cette faculté de formation des tissus

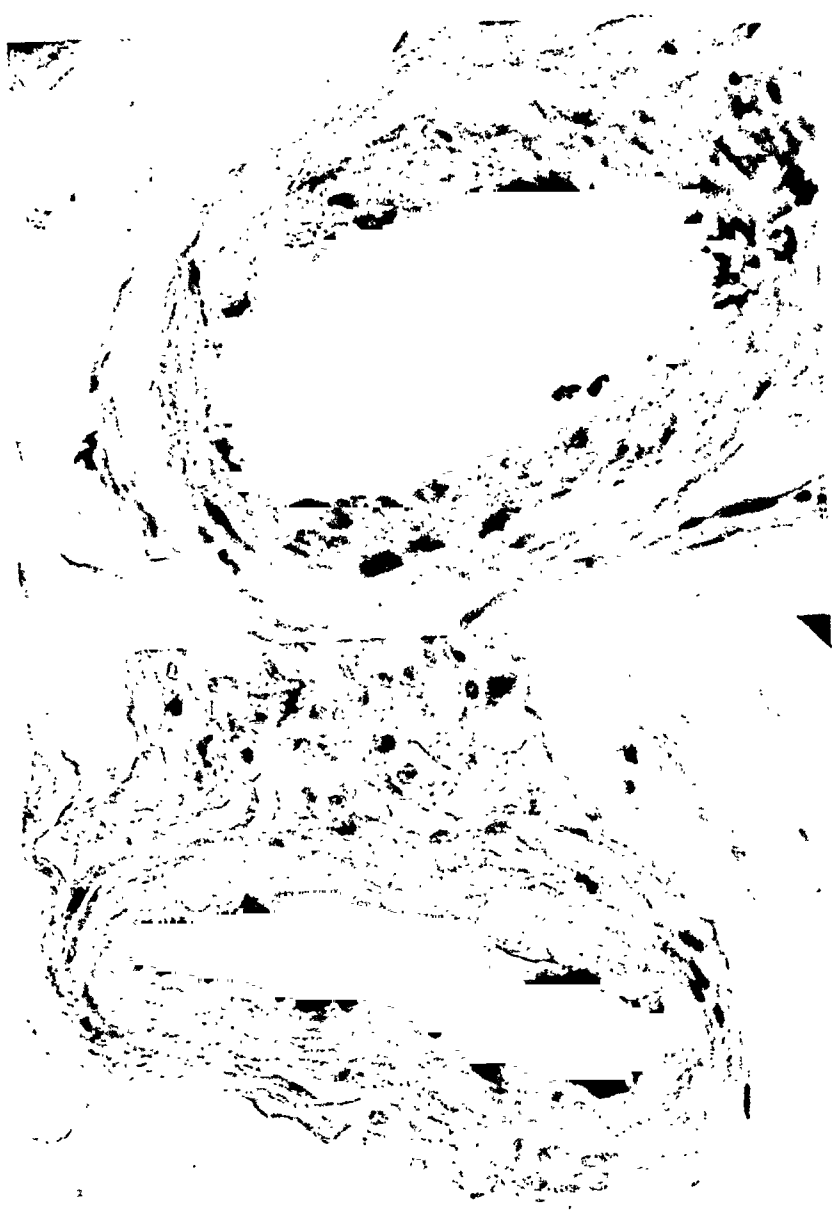


Photo 1.

Ulçère de l'estomac. Artères normales à distance de l'ulcère. L'artère représentée par la photo 2 se trouve à proximité de l'ulcère gastrique mais en dehors de la zone périulcéreuse. Artères injectées à l'encre de Chine.

Photo 2.



Photo 4.

Photo 3. Coupe au niveau de l'ulcère du même estomac que les deux photos précédentes. Endartérite oblitérante. Transformation progressive des cellules élastiques entre les couches de la média.

Photo 4. Formation de membranes élastiques entre les couches de la média.

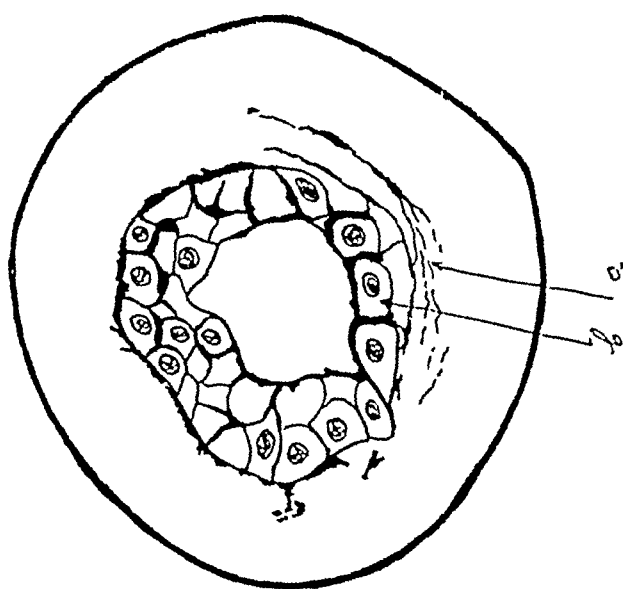


Photo 5.

Desin après coloration de l'élastine (Weigert résorcine) d'artères atteintes d'endartérite oblitérante. La fig. 5 montre l'apparition de cellules arrondies en dessous de l'endothéle, elles sont plus ou moins complètement entourées d'élastine (b) tandis qu'on voit s'en former également dans les couches plus excentriques (a).

La fig. 6 montre l'existence de l'élastine d'autant plus qu'on se rapproche de la périphérie de l'artère (a).

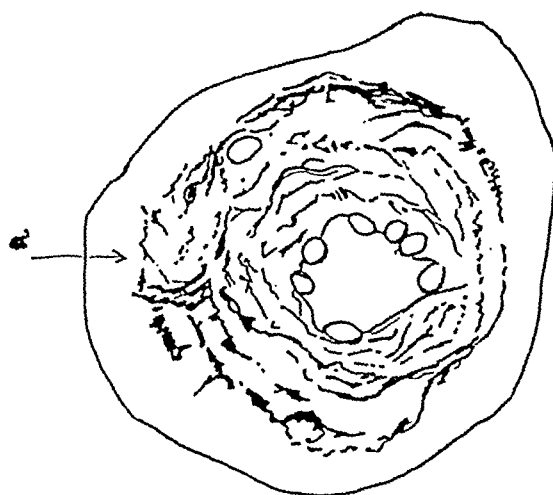


Photo 6.

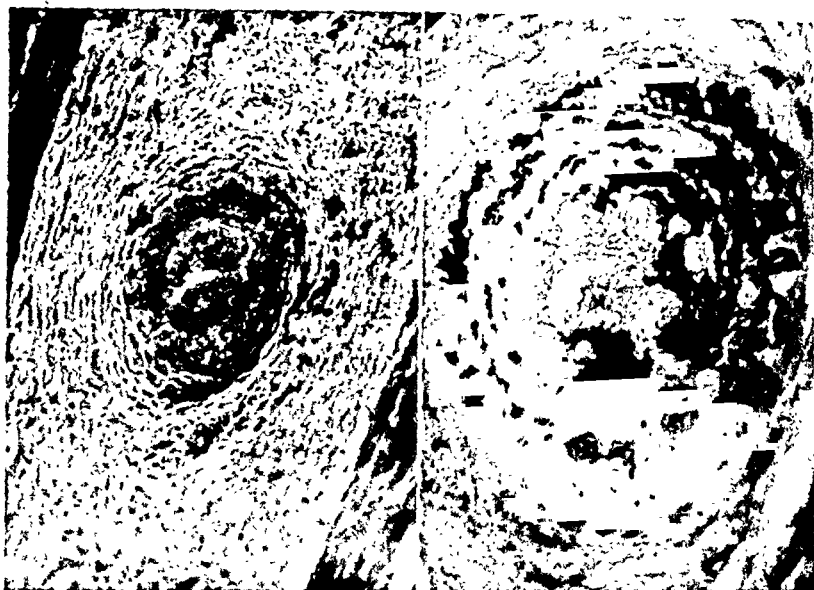


Photo 7.

Photo 8.

Artère située dans une forte couche de tissu conjonctif de néoformation inter-musculaire d'un estomac ulcéreux présentant une très forte augmentation de tissu conjonctif dans toutes ses couches.

Fort degré d'endartérite oblitérante (coloration trichrome Masson). La photo 8 représente la même artère, après coloration à la Weigert-résorcine. Couches multiples de membranes élastiques dont la plus forte est la plus externe. A certains endroits, la média a disparu ou presque: processus centrifuge. Remarquez l'aspect arrondi des cellules «intégrées» de la média. Les couches les plus internes ne présentent plus d'«imprégnation élastique». (Agrandissement différent pour les deux photos.)

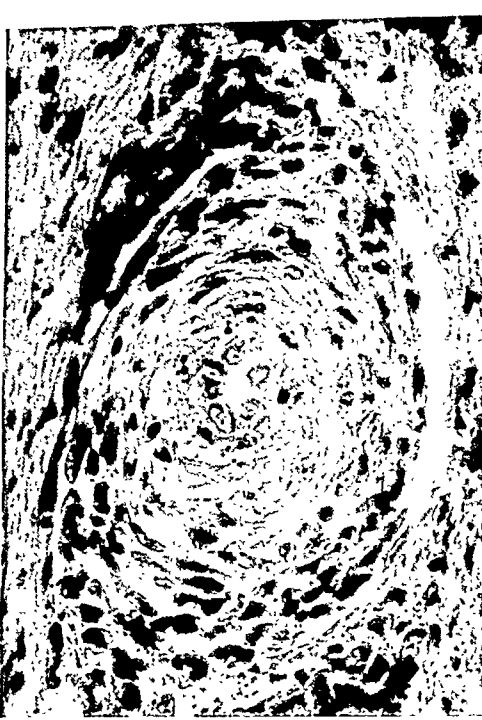


Photo 9.



Photo 10.

La même artère, coloration trichrome Masson (photo 9) et Weigert-résorcine (photo 10). Endartérite oblitérante. La partie centrale ne montre plus de membranes élastiques, la partie périphérique montre de l'élastine en couches d'autant plus épaisses qu'on se rapproche de la périphérie.

Stade plus évolué que les fig. 7 et 8.

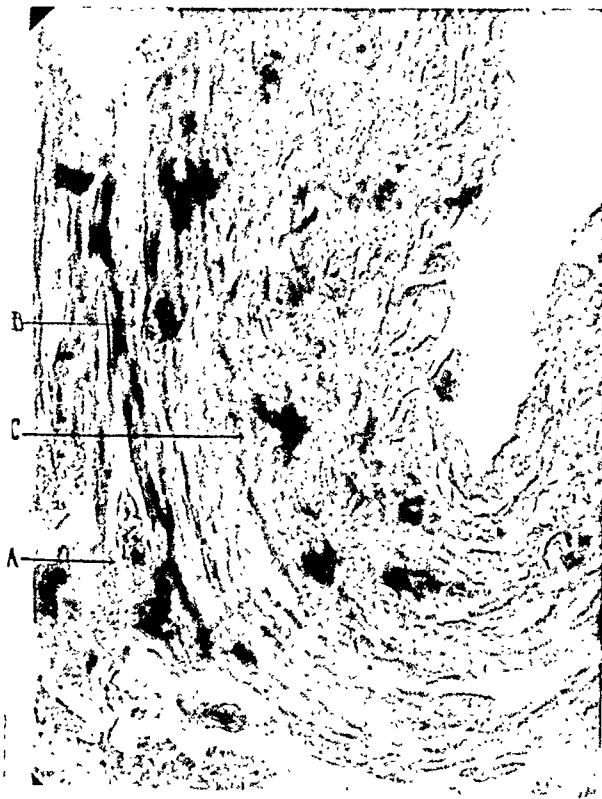


Photo 11.

Transformation des cellules de la média dont le caractère fibrillaire du protoplasme disparaît. Zone claire périnucléaire. Augmentation du tissu conjonctif entre les cellules musculaires. Arrondissement des noyaux au fur et à mesure qu'on se rapproche de la lumière. Remarquez les transformations en A. B. C.



Photo 12.



Photo 13.

Photo 12. Même artère que la photo 13 à un faible grossissement. Uniquement la couche «intimale» présente de l'élastine tandis que les couches de la média sont bien conservées.

Photo 13. Grande artère. La média est bien conservée et tranche nettement avec l'intima qui présente plusieurs couches poussant l'endothélium vers la lumière.

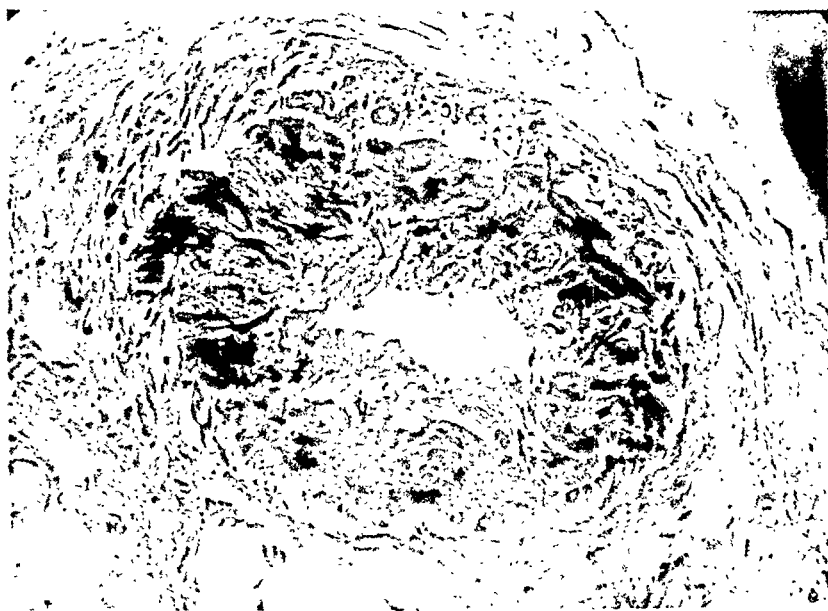


Photo 14.

Endophlébite. Couronne de tissu conjonctif sous-endothélial en connexion étroite avec le tissu conjonctif de la média et de l'adventice. Quelques cellules musculaires ont gardé leurs caractères propres, d'autres les ont perdus partiellement, ayant déjà subi la transformation conjonctive.

collagène et élastique. Le collagène et l'élastine — que nous ne savons repérer que par des méthodes de coloration, et au moment propice c.à.d. au moment où la substance fondamentale acquiert les propriétés physico-chimiques nécessaires à sa mise en évidence — apparaissent en dessous de l'endothèle puis progressivement dans les couches plus périphériques du vaisseau alors que les couches centrales perdent pendant ce temps le même caractère tout en constituant du tissu à prédominance cellulaire; la lumière se rétrécit. Dans un stade avancé, l'artère montre une lumière réduite entourée de plusieurs couches de cellules de »intima« qui dans leurs espaces intercellulaires ne présentent pas de tissu collagène ou élastique décelable par nos méthodes de coloration alors que les couches périphériques de la média montrent encore nettement une formation accusée de tissu conjonctif.

On a l'impression que l'excitant du tissu conjonctif prend son origine dans la lumière artérielle et se déplace vers la périphérie du vaisseau pour s'éteindre finalement et ne laisser subsister qu'une mince couche de la média. (Photos 9 et 10.)

Les grandes artères.

Les mêmes processus se retrouvent dans les grandes artères bien qu'il soit beaucoup plus difficile de les mettre en évidence et de suivre l'évolution, l'élastique interne semblant constituer une ligne infranchissable entre l'intima proliférante et la média.

La différence essentielle réside dans le fait que la formation de collagène et d'élastine se limite aux couches les plus centrales de la média tandis que plusieurs couches participent en même temps au processus, en cas d'endartérite oblitérante des vaisseaux de calibre moyen ou petit.

Si l'endartérite est prononcée, il existe un anneau riche en tissu conjonctif et en cellules. Plus le processus a évolué, plus le tissu conjonctif régresse et plus les éléments cellulaires prédominent.

Aux stades initiaux, tout le pourtour de la lumière n'est pas également entrepris par le processus. A certains endroits, l'endothèle est aplati présentant un aspect absolument normal et recouvrant l'élastique interne, l'intima étant difficilement visible. Le tissu conjonctif et élastique a légèrement augmenté entre les couches de la média. A d'autres endroits du vaisseau les cellules endothéliales semblent gonflées présentant des noyaux ronds à situation basale; le cytoplasme est clair. Le tissu conjonctif intercellulaire, en rapport intime avec le tissu conjonctif de la média

à travers les mailles de l'élastique interne, s'est fortement accru, entourant les cellules de toute part. Dans un stade plus avancé, on peut remarquer comment la couche la plus centrale de la média est délimitée du côté externe par une couche de tissu conjonctif collagène et élastique. On peut remarquer qu'à certains endroits se détache de l'élastique l'interne un faisceau de tissu élastique englobant une seule assise cellulaire de la média, les cellules gardant partiellement leurs caractères de cellules de la média. (Photos 12—13.)

Hueck pense que l'élastique interne forme de nouvelles lamelles élastiques vers la lumière du vaisseau. Mais pourquoi ces lamelles élastiques encerclent-elles exactement une couche cellulaire? D'où viennent ces cellules? Certainement pas de l'endothèle qui présente un aspect normal. Le passage de cellules de la média à travers les fenêtres de l'élastique interne n'a pas pu être observé. Tout comme dans les petites artères, la couche élastique la plus externe est la plus épaisse, elle est considérée comme l'élastique interne. En réalité elle constitue la couche la plus jeune. Tout comme dans les artères de calibre moyen et petit, le processus de néoformation de tissu conjonctif se fait excentriquement d'une façon progressive de sorte que la réaction est éteinte dans les couches centrales quand elles sont encore fort accusées dans les couches périphériques, la couche externe étant en pleine activité.

Dans les sinuosités des couches externes de l'élastique interne, les noyaux de la média se transforment: les noyaux fusiformes allongés prennent ci et là l'aspect d'un S dans lequel on peut observer trois parties. Les deux extrémités et la partie médiane de ce S ne sont pas situées dans le même plan. A un stade plus avancé, le S se divise effectivement en trois tronçons qui s'arrondissent et deviennent moins riches en chromatine. Ils ressemblent fortement aux noyaux de «l'intima proliférante». D'autre part, plusieurs noyaux de «l'intima proliférante» ont encore gardé l'aspect de ceux des cellules de la média; d'autres sont ellipsoïdes, de sorte qu'on peut rencontrer tous les stades intermédiaires «d'intégration des cellules de la média dans l'intima proliférante». Ces transformations des noyaux en constituent le premier stade.

Interprétation:

Si l'on examine les tranches successives obtenues par section perpendiculaire à la petite courbure après étalement du petit épiploon, on rencontre sur les coupes:

- 1) l'artère coronaire;
- 2) les branches pariétales de moindre calibre;
- 3) les branches marginales,
- 4) les multiples ramifications des trois précédentes.

On peut constater que:

a) on rencontre dans le voisinage immédiat de l'ulcère, la formation la plus accusée de tissu conjonctif qui va en diminuant au fur et à mesure qu'on s'éloigne de l'ulcère.

b) le degré de formation de tissu conjonctif est proportionnel au degré d'endartérite oblitérante c.à.d. que l'endartérite est d'autant plus prononcée que le tissu conjonctif a augmenté.

c) ce parallélisme n'est pas absolu: tous les vaisseaux ne sont pas atteints d'endartérite au même degré de sorte qu'on peut rencontrer sur une seule coupe divers stades du processus, indépendamment du calibre du vaisseau. L'hypertrophie de «l'intima» est alors surtout manifeste sur les grandes artères; les vaisseaux de calibre moindre ou moyen et dont la paroi n'est constituée que par quelques couches ne subissent qu'une hypertrophie relative tandis que les petites artères ne montrent que de la turgescence d'une seule ou de plusieurs couches sousendothéliales. Cette hypertrophie de l'intima peut ne se manifester que sur une étendue limitée et ne former qu'un bourgeon à l'intérieur de la lumière. Les grosses artères montrent une délimitation nette entre «l'intima hypertrophique» et la média constituée par une ligne ondulée: l'élastique interne. Le tissu conjonctif des deux couches communique par les fenêtres de l'élastique interne. Nous n'avons pas pu retrouver des cellules ou des noyaux qui seraient des éléments de transition entre les deux couches (Hueck).

Les petits vaisseaux peuvent être atteint d'un degré très prononcé d'endartérite.

C'est par l'étude des artères de calibre moyen, où l'élastique interne est moins nettement visible, que la transition de la média à «l'intima» peut être suivie le mieux, tant en dehors qu'au niveau du fond de l'ulcère même.

d) le tissu conjonctif de néoformation est fort développé dans la sousséreuse, moins fort dans les couches musculaires, un peu moins dans la sousmuqueuse; même la muqueuse présente du tissu conjonctif en quantité accrue, tout au moins si l'estomac présente dans son ensemble un fort accroissement du tissu conjonctif (en cas d'ulcère du ventricule).

e) la partie sousendothéliale hypertrophique ne présente pas le

même aspect anatomique dans les vaisseaux de même calibre, c.à.d. que certaines artères montrent un fort développement de tissu conjonctif collagène tandis que d'autres en montrent fort peu, le tout suivant le degré d'évolution de l'endartérite oblitérante.

f) Si on examine des coupes de fragments prélevés à distance de l'ulcère mais où il y a encore un faible accroissement de tissu conjonctif, donc encore situés dans la zone d'induration périulcéreuse, on constate que les vaisseaux artériels de calibre moyen présentent, en dessous de l'endothèle, qui a un aspect normal, une ou plusieurs couches de cellules épithéloïdes aux noyaux ronds. On peut y trouver toutes les formes de transition entre les cellules musculaires fibrillaires et les nouvelles formations dont les noyaux de la média sont parfois réniformes, sphéroïdes ou ronds.

Quand nous laissons défiler devant notre esprit toutes les images des coupes successives d'une seule artère ou de toutes les artères des endroits éloignés de l'ulcère jusqu'au niveau de l'ulcère, il nous paraît qu'on peut se représenter le processus de l'endartérite progressive de la façon suivante: le premier stade est constitué par la turgescence d'une ou de plusieurs assises cellulaires en dessous de l'endothèle; ces cellules acquièrent un aspect épithéloïde. Ceci se rencontre dans les petites artères. Cet endothèle peut présenter au début un aspect normal mais quand le processus progresse, ses noyaux peuvent devenir turgescents.

En harmonie avec l'accroissement général de tissu conjonctif on voit apparaître, progressivement et en quantité variable suivant le calibre du vaisseau, du tissu conjonctif collagène et élastique entre les assises cellulaires dont il est question tandis que les cellules musculaires typiques de la média se transforment progressivement. Cette imprégnation par l'élastine et la formation de tissu collagène n'est pas complète c.à.d. que certaines cellules musculaires transformées qui prennent un aspect épithéloïde ne sont entourées que très incomplètement par du tissu conjonctif.

Il paraît improbable que ces cellules pourraient être d'origine endothéliale puisque nous n'avons pas pu retrouver de formes de transition.

Suivant Hueck, il persiste de la substance fondamentale indifférente mésenchymateuse dans la paroi artérielle, elle ne se laisse pas imprégner par les méthodes de coloration habituelles mais son rôle ne peut pas être sousestimé. En cas d'endartérite, cette substance mésenchymateuse intercellulaire acquiert à nouveau sa

propriété embryonnaire de former du tissu collagène et élastique non seulement dans l'artère mais dans toute la paroi gastrique. La démonstration en est fournie par le grand espace — difficile à colorer — entre les cellules musculaires de la média qui participent déjà au processus ou qui sont sur le point d'y participer. Le collagène et l'élastine apparaissent comme enrobés dans cette substance fondamentale. *C'est l'ulcère qui constitue le facteur causal du réveil de cette propriété embryonnaire du tissu fondamental intercellulaire mésenchymateux.*

L'accroissement irrégulier du tissu conjonctif intercellulaire fait comprendre la disposition désordonnée des cellules épithéloïdes.

Si on examine la *disposition des fibres élastiques*, on remarque que:

1) en cas d'endartérite oblitérante on trouve dans les artères de calibre moyen ou petit des formations élastiques fragmentaires aussi fortement imprégnées que l'élastique interne avec laquelle elles sont en connexion intime mais situées dans les couches plus excentriques.

2) partant de l'élastique interne, on voit de fortes fibres élastiques s'insinuer entre les cellules de la première rangée cellulaire de la média et les entourer sur leurs faces latérales et centrales.

3) Dans certaines artères, *en dehors* de l'élastique interne, on trouve de grosses lamelles élastiques; elles sont en rapport avec les lamelles sousjacentes; elles sont séparées l'une de l'autre exactement par une couche cellulaire, mais elles sont plus excentriques.

Conclusion: L'endartérite oblitérante trouve son origine dans un regain de la propriété embryonnaire de la substance fondamentale intercellulaire mésenchymateuse de la média qui consiste à former du tissu conjonctif collagène et élastique, englobant des cellules de la média dans «l'intima proliférante». Ce processus d'intégration dans «l'intima» des cellules de la média a un début central, il progresse excentriquement, pouvant s'arrêter à un moment déterminé ou pouvant passer par toutes les couches de la média de telle sorte que cette faculté d'imprégnation du tissu collagène et élastique a cessé dans les couches centrales au moment où les couches externes sont en pleine activité. Les artères de calibre moyen se prêtent le mieux à cette étude.

Considérations:

Si nous examinons les données de la littérature, nous devons conclure qu'il n'existe pas toujours une même modalité de réaction

de la paroi artérielle vis-à-vis d'une excitation donnée. En effect on ne constate parfois que des réactions de l'endothéle ou des couches sousendothéliales, mais parfois aussi de toute la paroi artérielle.

Le facteur étiologique est inconnu: on a incriminé des causes mécaniques, chimiques, toxiques, allergiques.

En ce qui concerne l'endartérite oblitérante des artères gastriques, en cas d'ulcère gastrique, il faut considérer l'ulcère comme le facteur étiologique (nous ignorons la nature de cette excitation). Nous en trouvons la preuve dans le fait que l'endartérite oblitérante des artères gastriques s'étend exactement aussi loin que le bourrelet d'induration périulcéreuse: ceci découle de l'examen d'estomacs réséqués et injectés dans les artères par de la substance contrastante au R. X. ainsi que de l'examen microscopique. D'autre part l'intensité de l'endartérite oblitérante est proportionnelle à celle de la gastrite concomitante de l'ulcère; or, cette gastrite concomitante dépend, quant à sa localisation et son intensité, directement de la localisation de l'ulcère.

Sous l'influence de cette excitation inconnue, partant de l'ulcère, les cellules de la média rétrocedent en différenciation et redeviennent des fibroblastes. Rozinek a décrit les étapes successives de la transformation de fibroblastes de l'adventice en cellules typiques de la média. De même Arnold, V. Möllendorf, Renaut et Dubreuil, Benninghof et Stieve acceptent comme possible la transformation de cellules conjonctives en cellules musculaires.

L'endartérite oblitérante est un processus de régression avec augmentation considérable du tissu conjonctif collagène, élastique et de la substance fondamentale intercellulaire amorphe.

Il est cependant possible que l'hyperplasie de «l'intima» ne se manifeste que sur une partie du pourtour de la lumière artérielle et donne lieu à la formation d'un bourgeon conjonctif qui peut être en rapport, à travers la média, avec le tissu conjonctif de l'adventice.

L'endophlébite.

Watzka a décrit certaines formations de barrage »Sperrvorrichtungen» provoquant un rétrécissement de la lumière des vaisseaux et qui seraient capables de jouer un rôle régulateur de la circulation.

Ces formations se rencontrent rarement et il nous semble qu'elles sont incapables d'influencer sérieusement le débit du vaisseau.

Elles sont d'ailleurs peu étendues. Nous les avons observé, bien que très exceptionnellement, sur des veines qu'il faut considérer comme normales à tout autre point de vue.

En cas d'endophlébite au cours de l'ulcère de l'estomac, nous avons observé deux modalités. La première consiste dans la formation d'un bourgeon sessile de tissu conjonctif recouvert d'un endothélium. Cet endothélium présente un aspect normal. Le bourgeon est formé de tissu conjonctif collagène pourvu de noyaux polymorphes, irrégulièrement disposés. Ces bourgeons conjonctifs sont en connexion intime avec le tissu conjonctif autour du vaisseau et ce au moyen de ponts conjonctifs traversant la média; ces noyaux sont très distincts et à direction perpendiculaire à ceux de la couche musculaire.

D'autres formations bourgeonnantes conjonctives sont en connexion très étroite avec le tissu conjonctif de la média de la veine. Par ci, par là, dans ces bourgeons conjonctifs, on rencontre quelques cellules musculaires lisses.

Une autre modalité de l'endophlébite consiste en une prolifération uniforme et massive de tissu collagène tout autour de la lumière de la veine, recouverte par un endothélium normal. Cette formation conjonctive part distinctement du tissu conjonctif de la couche musculaire de la veine. Elle présente quelques cellules musculaires lisses dispersées sans ordre. (Photo 14.)

L'endophlébite n'est nullement aussi accusée en cas d'ulcère de l'estomac, que l'endartérite.

Il semble que le processus qui se produit dans la paroi de la veine soit identique à celui que nous avons décrit dans la paroi de l'artère.

Alors que le processus d'endartérite oblitérante va jusqu'à l'occlusion complète de l'artère, le processus d'endophlébite ne constitue jamais une aussi forte diminution du calibre de la veine.

L'endophlébite démontre d'une façon plus démonstrative que dans l'artère, que toutes les couches participent ou peuvent participer au processus. Ici encore nous remarquons la différence entre la structure de l'artère et celle de la veine. Cette dernière présente, normalement un enchevêtrement de tissu conjonctif et de fibres musculaires, le tout couvert, à l'intérieur, d'un endothélium. Le facteur déterminant et encore ignoré de l'endophlébite semble frapper circulairement autour de lui, à point de départ central. Le résultat en est que nous pouvons observer dans cette veine, à un stade donné, une couronne de tissu conjonctif, pour préciser

davantage, disons plutôt qu'elle acquiert les propriétés tinctoriales du tissu conjonctif collagène, en rapport étroit avec le tissu conjonctif de la média et de l'adventice. Mais ce qui est plus intéressant encore, c'est que nous pouvons observer que certaines cellules musculaires sont partiellement transformées en cellules conjonctives. Partiellement c.à.d. que certaines cellules musculaires ont perdu leur caractère fibrillaire; leurs noyaux, des oblongs originairement, sont devenus ronds ou sphériques, avec tous les stades intermédiaires. De plus, il est des cellules musculaires qui sont divisées nettement en deux parties bien distinctes, correspondant au cercle de transformation conjonctive c.à.d. que la partie de la cellule musculaire située en dedans de ce cercle a subi la transformation conjonctive alors que la portion située en dehors du cercle a gardé toutes les propriétés et caractéristiques de la cellule musculaire.

Ces cellules de transformation et de transition se rencontrent surtout à la ligne de séparation de la média conservée et de «l'intima proliférante». Ceci anéantit l'interprétation de Thoma qui attribue une origine endothéliale à ces cellules transformées. D'ailleurs l'endothélium, en tous points normal, recouvre la couche néoformative.

La transformation progressive des noyaux cellulaires de la couche proliférante vers le centre démontre que le processus est centrifuge (diminution progressive de la chromatine, perte progressive du caractère réticulaire du noyau, à mesure qu'on se rapproche de la lumière du vaisseau).

Summary.

A distinction must be made between the small arterioles, the middle-sized arteries and the big arteries.

The first ones present some degree of turgescency of their cells. The middle-sized arterioles acquire an embryonic propriety in their collagen and elastic connective tissue: the fundamental intercellular substance becomes active and productive; it gets more easily impregnated of coloring substances used in histology, it proliferes while the muscular cells of the media transform themselves into a sort of connective cells which are badly delimited; their nuclei are round, ovoid or polymorph. This connective transformation is a centrifugal one. The complete closure of the lumen of the artery is the final result.

In the big arteries the obliterating process is essentially marked by a proliferation of the connective tissue under the endothelium and in the media. The elastica interna, however, forms a barrier that limits more effectively the different layers of the artery.

The vene has the same characteristics as the artery when it makes a process of endophlebitis; however, this process occurs less frequently. One may observe the progressive transformation of the muscular cells into connective ones. There is a parallel proliferation of the connective tissue.

Literature.

H. Bredt. Entzündung und Sklerose der Lungenschlagader. Virchows Archiv, vol. 308, p. 60, 1941. — Coronini, Oberson. Neue histologische Ergebnisse bei Endophlebitis obliterans hepatica. Virchows Archiv, vol. 298, p. 251, 1937. — Dietrich Schroeder. Abstimmung des Gefäßendothels als Grundlage der Thrombenbildung. Virchows Archiv, vol. 274, p. 425, 1930. — M. Fossel. Über das Vorkommen von dickwandigen Arterien in der Lunge bei gleichzeitiger Hypertrophie der Lungensmuskulatur. Virchows Archiv, vol. 309, p. 701, 1942. — J. L. Mac Kelvey, H. E. Mac Mahon. A study of the lesions in the vascular system in fatal cases of chronic nephritic toxæmia of pregnancy. Malignant nephrosclerosis. Surg., Gyn., Obst. p. 1, 1935. — J. König. Über eine fatale Form der Endophlebitis hepatica obliterans. Virchows Archiv, vol. 311, p. 527, 1943. — R. Lindenberg, H. Spatz. Über die thromboendarteritis obliterans der Hirngefäße. Virchows Archiv, vol. 305, p. 530, 1939. — A. J. Linzbach. Über generalisierte Gefäßverkalkungen bei einem Fall von gleichzeitiger knöcherner Stenose der Trachea und der Bronchien. Virchows Archiv, vol. 308, p. 629, 1942. — M. Masugi-Ya-Shu. Die Diffuse Sklerodermie und ihre Gefäßveränderung. Virchows Archiv, vol. 302, p. 39, 1938. — H. Merkel. Über verschlussfähige Bronchialarterien. Virchows Archiv, vol. 308, p. 301, 1941. — C. Papp. Die arteriosklerotische Leber. Virchows Archiv, vol. 290, p. 551, 1933. — W. Rintelen. Über die experimentelle allergisch-hyperergische Arteritis. Virchows Archiv, vol. 299, p. 629, 1937. — Rössle. Zum Formenkreis der rheumatischen Gewebsveränderungen mit besonderer Berücksichtigung der rheumatischen Gefässentzündungen. Virchows Archiv, vol. 288, p. 780, 1933. — M. Rozynek. Untersuchungen über die Differenzierung der Blutgefäße in Angiomen. Virchows Archiv, vol. 307, p. 678, 1940. — Fr. Wiese. Über Thromboendarteritis obliterans der Lungenarterien. Frankf. Zeitschrift. J. Pathol. vol. 49, p. 153, 1936. — Siegmund, H. Untersuchungen zur Pathogenese der Endokarditis, insbesondere der Frühveränderungen. Virchows Archiv, vol. 290, p. 3, 1933. — Cfr. Henke-Lubarsch. Handbuch der Spez. Pathol. Anat. u. Histol.

From the Second Department, Kommunehospital, Copenhagen.
(Physician-in-Chief: H. Heckscher, M. D.)

The Thymol Reaction as a Liver Test.¹

By

INGER-LOUISE MARNER.

(Submitted for publication October 14, 1947.)

Introduction.

While working on the serum colloidal gold reaction in 1944, Maclagan (1) observed that thymol produced a turbidity, and sometimes precipitation, with serum from patients with parenchymatous liver lesions. The reaction was noted in 120 out of 130 cases of acute hepatitis, in 13 cases of liver cirrhosis; but in 38 cases of obstructive jaundice there were only three reactions, and these slightly positive. Maclagan believed that the reaction would play an important rôle in the differential diagnosis of jaundice, and that it would be useful as an indicator of the degree of liver damage. If the test satisfies these two desiderata, it must be said to fulfil the two most important requirements of a liver test.

In order to ascertain the cause of the turbidity, Maclagan (2) analyzed the sediment and found that a protein-thymol-phosphor-lipoid complex was involved, and that the deciding factor must have something to do with the plasma globulin fraction.

Subsequent investigations by Recant, Chargaff and Hanger (3) and at the Central Laboratory at Upsala (4) verified Maclagan's supposition; but the question of the actual nature of this special property of the globulin fraction has not yet been settled. Nevertheless, on the background of recent experiments with electrophoresis Cohen & Thompson (5) consider that a positive thy-

¹ Amended form of paper delivered on Febr. 28th 1947 before the 250th meeting of the Danish Society of Internal Medicine.

mol reaction must be due to abnormalities associated with the β -globulins.

In order to measure the degree of turbidity in positive tests Maclagan made use of a comparator with gelatin standards such as those employed for the quantitative determination of the protein content in urine (6, 7).

In 1946 this method of measuring was modified by Shank & Hoagland (8), who found the preparation of the gelatin standards difficult. Their method was to read the degree of turbidity in the spectrophotometer, using suspensions of barium sulphate as standards. The standards were obtained by adding varying quantities of 0.2-n sulphuric acid to varying quantities of a barium sulphate suspension (3 ml 1-n barium chloride diluted to 100 ml with 0.2-n sulphuric acid at 10°). These standards provide degrees of turbidity which are almost equivalent to the gelatin standards employed by Maclagan.

With the object of further standardizing the method quantitatively, Ley, Lewis & Davidson (10) next introduced photo-electric readings. Their »thymol number» is the number of ml. of the barium sulphate suspension required to give the standard the same degree of turbidity as the serum-thymol mixture concerned.

The thymol test has now been adopted extensively for clinical use in England, the United States and Scandinavia. Most of the larger publications so far are concerned with a comparison of the thymol reaction and Hanger's liver function test (cephalin-cholesterol flocculation) (3, 4, 9). According to these investigations, the thymol test seems to be less sensitive to parenchymal lesion, whereas it is more suitable as an aid to differential diagnosis between parenchymatous and obstructive jaundice. Furthermore, there seems to be a difference in the chemistry of the two reactions.

Technique.

The thymol test is performed quickly and easily. The thymol buffer (pH 7.8) is prepared in this way:

- 500 ml distilled water
- 1.03 g sodium barbitone
- 1.38 g barbitone
- 3 g pulverized thymol.

Heat the solution to boiling point, then shake vigorously and cool; it then becomes turbid. Add further a knife-point of thymol, shake well, then let it stand till next day at room temperature. Shake the solution again and filter. The clear solution is best stored in a cool place, as otherwise it soon becomes turbid again, *i. e.* in about 14 days.

Mix 0.05 ml serum with 3 ml thymol buffer and leave the mixture for half an hour at room temperature.

It having proved impossible for me to prepare Maclagan's gelatin standards according to the directions (6, 7), my readings were not taken according to Maclagan's method, but by means of the Pulfrich photometer after directions from Lehmann in Sweden. Filter S. 72 and 1 ml cuvettes are used. The reading is made against distilled water, which gives the value of 0. The thymol value is given direct as the extinction number. By this means we seem to have obtained an exact standardization of the method, quantitatively, the photometer values being practically constant.

Own Investigations.

The principal sources of error in the thymol test are: 1) considerable hemolysis, 2) lipemia, 3) bacterial growth.

Lipemia is best avoided by taking serum from fasting patients; however, a test of 14 healthy people and liver patients showed that a cup of tea and a rusk do not alter the thymol value — a fact which agrees with earlier investigations into the alimentary increase of blood fat (Bing & Heckscher, Blix) (11, 12).

Thymol Values.

After testing 40 healthy young men and women the mean value was found to be 0.07 ± 0.02 . In consequence of this it seems practical to divide the thymol values into three groups:

- 1) Normal thymol values: $0.07 \pm 3 \times 0.02 = 0 - 0.13$.
- 2) Doubtfully positive: $0.13 - 0.07 + 4 \times 0.02 = 0.13 - 0.15$.
- 3) Positive: >0.15 .

The highest thymol values observed in cases of liver damage were about 1.5.

Thymol Test in Various Cases of Jaundice.

The thymol test was made on 107 jaundice patients¹ on an average once a week while the disease lasted. Of these 107 patients:

55 had acute hepatitis

27 had subchronic or chronic hepatitis

5 had metastatic cancer of the liver

20 had obstructive jaundice.

A case was regarded as one of acute hepatitis when the patient recovered and the total course, as far as could be ascertained, had not extended over three months and when subsequent examination revealed no permanent liver lesion. More protracted cases, and those with a subsequently demonstrable liver lesion were classified as subchronic or chronic hepatitis. The latter group includes all cases terminating fatally, even when this termination came within three months of the onset of the disease, because histological examination of the liver tissue disclosed changes otherwise observed only in chronic cases.

The thymol reaction was positive in 50 out of the 55 cases of acute hepatitis, *i. e.* in 91 %, and in most of these cases repeatedly. All the 27 patients with chronic hepatitis had a positive thymol reaction. Of the five patients with metastatic cancer of the liver, three gave a positive, two a negative reaction. In the examination of the 20 cases of obstructive jaundice, all verified by operation or post-mortem, the thymol test was positive only in two instances, in both of these, however, the reaction was weakly positive. The first of these patients who only once presented positive thymol reaction (0.19), had cholelithiasis which may have caused an obstruction. The other patient died and the post-mortem revealed cholelithiasis, cholangitis and incipient formation of abscess in the liver; in the only test made in this case the value was found to be 0.22.

Fig. 1 provides a collective survey of the values found.

Quantitative Relationship between Thymol Test and Icterus Index.

On examining simultaneously the thymol values and the icterus index in cases of acute hepatitis and comparing these finds in the different cases (Fig. 2) it seems difficult to ascertain any

¹ Patients in the medical and surgical departments of the Kommunchospital.

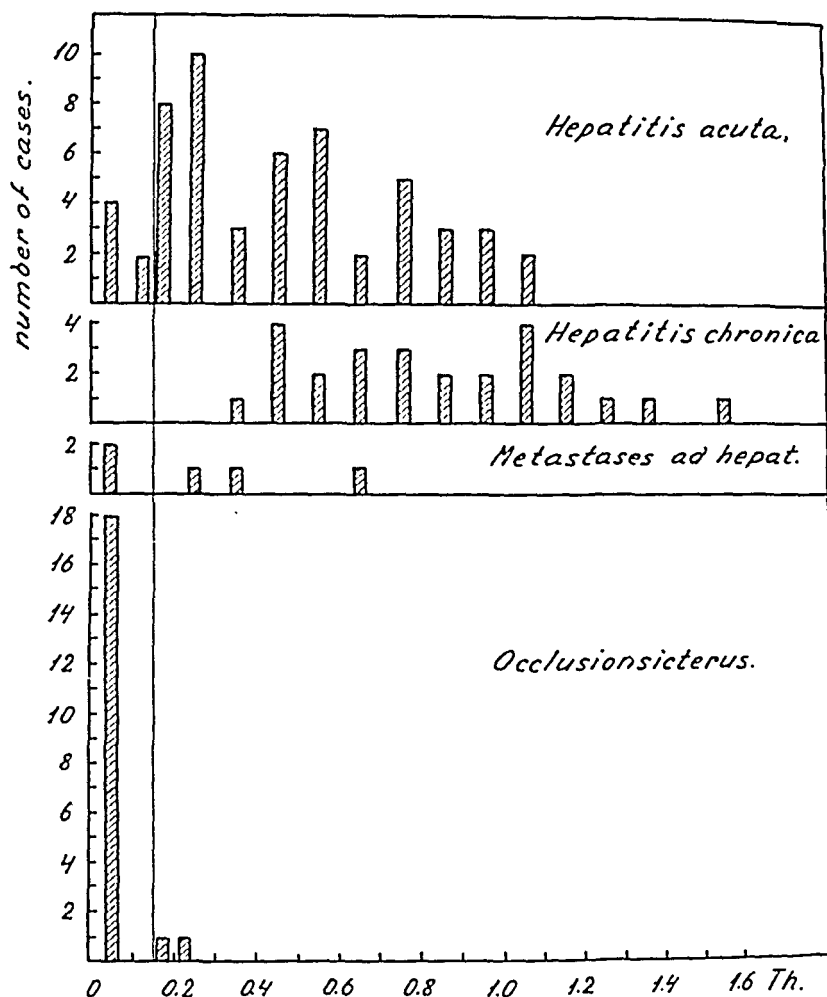


Fig. 1. All examined cases grouped according to the maximum thymol values (Th) found (the height of the columns indicates the number of cases).

direct quantitative correlation, as very high icterus index can be found together with low thymol values, whereas low icterus index can be found simultaneously with high thymol values. (Fig. 2.)

In most cases of acute hepatitis, however, the maximum icterus index and the maximum thymol value coincided in time.

In cases of chronic hepatitis the icterus index may fluctuate and at times reach quite normal levels, whereas the thymol values remain distinctly high all the time, but as a rule the thymol value is highest in the icteric periods.

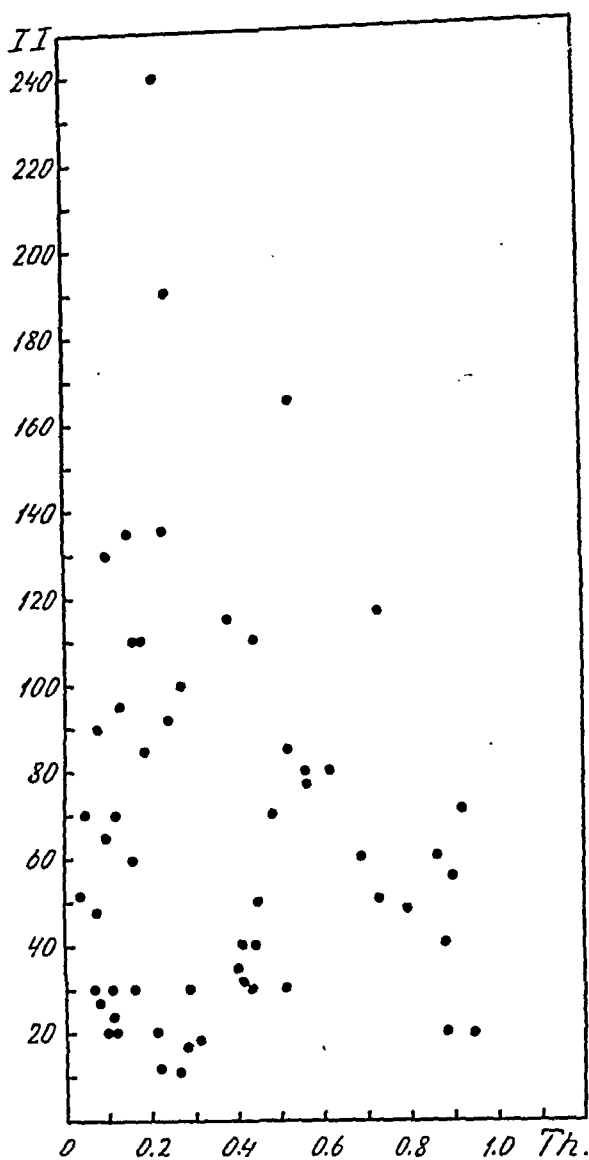


Fig. 2. The relation between the highest icterus index (II.) found for every patient and the thymol value (Th.) found simultaneously in 55 cases of hepatitis acuta.

Thymol Test in the Preicteric Stage.

It is of great interest to see whether the thymol test becomes positive before, at the same time or later than the icterus index. Unfortunately, I can present only one instance where the thymol test was made before icterus appeared. It was made two days

before icterus was observed, and the reaction was slightly positive (0.16). In another instance the test was made on the day when icterus was observed. It was then positive (0.20) (icterus index $II = 12$).

Twenty-five cases of acute hepatitis were tested within the first eight days after the appearance of the jaundice. Of these, 21 had an increased thymol value, one was increased only after three weeks, and three remained negative throughout the entire duration of the attack. Thus it may be said that in the great majority of cases of acute hepatitis the thymol test is positive within the first eight days of jaundice appearing.

Thymol Value as a Test of the Course of the Disease.

If cases of acute or chronic hepatitis are followed by means of thymol tests about once a week, it is possible to obtain a certain impression of the course of the disease from the curve, usually quite a steady one, that can be plotted (fig. 3).

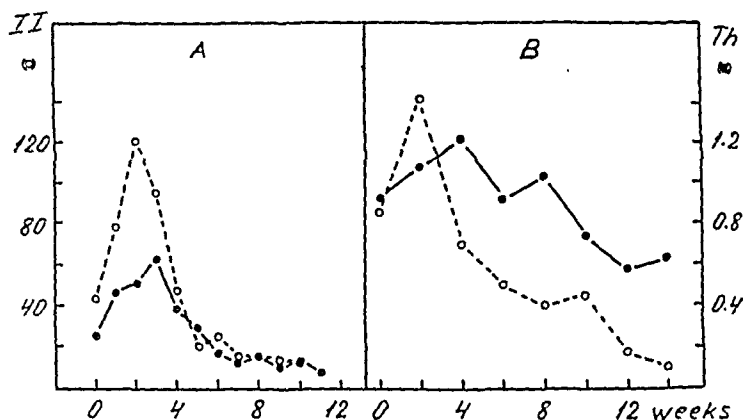


Fig. 3. Specimen thymol curve during the course of a typical attack of acute hepatitis (A) and chronic hepatitis (B), showing the icterus index found simultaneously.

————— = thymol value (Th.).
 - - - - - = plasma colour value (Pl.).

In 23 out of 33 cases of acute hepatitis the thymol reaction was still positive after the icterus index had fallen to normal. At this juncture the Takata-Ara test, which had been positive at one time or another in 23 out of the 55 cases of acute hepatitis, had become negative in all cases but one.

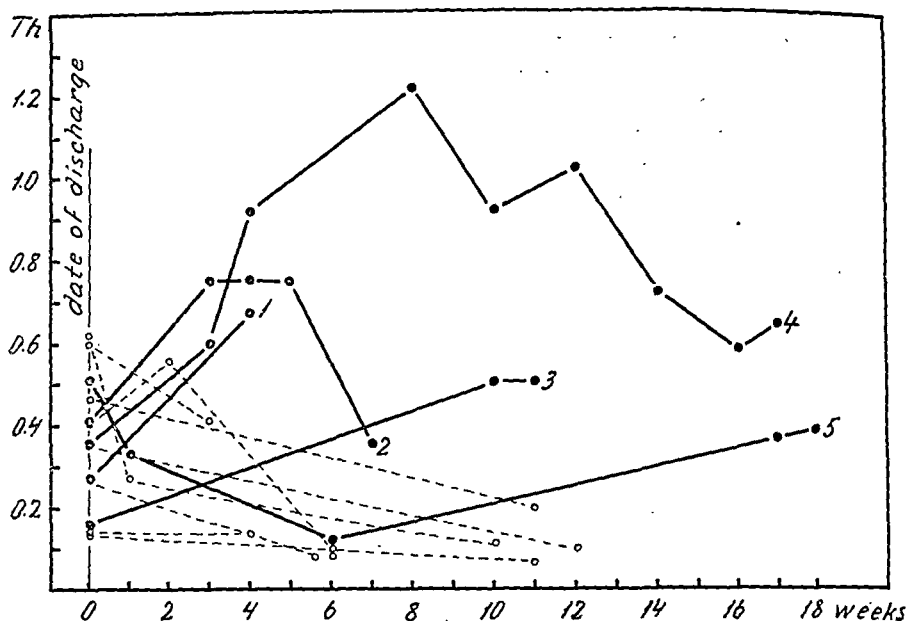


Fig. 4. Thymol value curve plotted from the after-examination of 12 patients discharged with a raised thymol value. Five curves are singled out (the continuous curves).

found to have risen to 0.50. TA., which formerly was negative, is now weakly positive. II. 6.

No. 4 (♀ b. 10/7-96) on discharge had Th. 0.36, TA. moderately positive, II. 7, but at the time of the afterexamination three weeks later there were nausea, epigastric oppression and slight jaundice. Th. had risen to 0.66, whereas TA. was negative. About eight days subsequent to the after-examination the patient was rehospitalized with II. 85, Th. 0.93, TA. weakly positive.

No. 5 (♀ b. 3/12-21) on discharge had Th. 0.51, TA. negative, II. 7. At the after-examination six weeks later Th. had fallen to 0.13 and there was only slight lassitude; twelve weeks later, however, the patient was unwell, temperature 38°, epigastric oppression, nausea, slight jaundice of the sclerae. At this juncture Th. was 0.38, TA. weakly positive.

The suggestion deriving from these observations is that persistent positive thymol reaction in presumably recovered patients with hepatitis may indicate a protraction of the disease apt to lead to chronic impairment of the liver.

Specificity of the Thymol Reaction.

It is presumable that most of the observations enumerated above may be taken as a sign that in the positive thymol reaction we have a symptom of liver lesion due to hepatitis. In order to

clarify this symptom — or rather its reliability — I have made thymol tests on 396 patients¹ with various internal diseases, and on 29 women in the last three months of pregnancy². Six of the latter had albuminuria.

Similar tests were made earlier by Maclagan, who observed positive reactions in 21 out of 217 patients.

Of the 415 patients referred to, 37 gave a positive thymol reaction and 19 a doubtfully positive reaction.

The following table shows how these reactions were distributed among the various disease groups.

Table.

Thymol Values of Patients with Various Internal Diseases.

	Diagnosis	Number of Cases	Thymol-positive & Doubtfully Positive Cases	Thymol-negative Cases
Gastric and intestinal diseases	Ulcus ventriculi	24		5
	» duodeni			10
	Cancer ventriculi			4
	» intestini			4
Respiratory diseases	Helminthiasis	43		1
	Pneumonia		1	23
	Bronchitis chron.		2	5
	Asthma bronchiale		(1)	2
	Pleuritis exsudativa		1	5
Heart diseases	Tuberculosis pulmonis	7	(1)	2
	Stasis hepatis		(2)	5
Joint diseases	Febris rheumatica	25	2	9
	Polyarthrit. chron.		5 (2)	6
	Periarthrosis			1
Kidney diseases	Pyelonephritis chron.	7		5
	Glomerulonephritis chron.			2
Blood diseases	Anemia perniciosa	6	1	3
	Leucosis lymf. chron.			1
	Myelomatosis multiplex		1	
Endocrine diseases	Morbus Basedowii	15		9
	Myxoedema		1	1
Nervous diseases	Diabetes mellitus	6		4
	Sclerosis disseminata			2
	Apoplexia cerebri			4
Diseases of internal genitals	Cancer uteri	5		3
	» prostatae			2

¹ Patients at the Kommunehospital and the Blegdam Hospital.

² Pregnant women in the Rigshospital Lying-In Dept. A.

Table. (Cont.)

	Diagnosis	Number of Cases	Thymol-positive & Doubtfully Positive Cases	Thymol-negative Cases
Special infectious diseases	Gastroenteritis acuta		(1)	29
	Febris typhoidea			6
	Paratyphus		(1)	4
	Catarrhalia		(3)	2
	Tonsillitis acuta		2 (3)	86
	Mononucleosis infectiosa	248	18 (5)	11
	Diphtheria			6
	Scarlatina			14
	Morbilli + Pneumonia		2	8
	Parotitis epidemica			1
	Erysipelas			17
Pregnancy	Meningitis			29
	÷ Albuminuria	29		23
	+ " "		1	5
Totals		415	36 (19)	359

The number of positives among mononucleosis patients is remarkably high, 18 out of 34. The cause may be that the liver is rather often suffering in mononucleosis (Thomsen, S. (14), Bang, J. & Wanscher, O. (15)). In his investigations with electrophoresis in cases of infectious hepatitis Martin (16) discovered in 1946 that the electrophoretic finds are not specific for infectious hepatitis, similar shadows being observed in mononucleosis and lupus erythematosus.

The control investigation into the thymol test on patients with various diseases that cannot be grouped as hepatitis, obstructive jaundice or cancer of the liver, shows that sometimes such diseases are capable of inducing the blood changes that are demonstrable by means of the thymol test. Whether this involves liver damage — which perhaps is most probable — or other things remains uncertain for the present. However, this limitation of the significance of the thymol test is of less practical importance compared with the value of the test as an adjuvant to differential diagnosis between hepatitis and obstructive jaundice.

Epicrisis.

I shall now summarize the main points of the foregoing.

An outstanding feature of the thymol test is its rapidity and simplicity, and quantitatively it is standardized when the photometer is used for the reading. Consequently it is an easily applied laboratory test.

The results of earlier investigators suggest that the thymol reaction reflects an abnormal condition in the globulins of the blood, maybe abnormal conditions in the β -globulins.

The thymol reaction is most often, and most constantly, positive in cases of hepatitis, and when repeated, provides a numerical series which indicates the course of the disease. In contrast, the reaction is mostly negative or weakly positive in cases of obstructive jaundice. The test is therefore of importance as circumstantial evidence in the differential diagnosis between hepatitis and obstructive jaundice.

In some instances the thymol reaction remains positive after the acute hepatitis — judged by other clinical methods — has subsided.

Agreeing with the experience of Kunkel & Hoagland and the investigations of the Hospital of the Rockefeller Institute, these observations suggest that in cases of hepatitis prolonged positive thymol reaction after the subsidence of other clinical symptoms must be regarded as a sign of persistent impairment of the liver.

Summary.

1) The author briefly describes the technique of the thymol test with readings on the Pulfrich photometer.

2) 107 patients with jaundice were subjected to the thymol test. It was found to be positive in 91 % of the cases of acute hepatitis, in 100 % of cases of chronic hepatitis, and in 10 % of cases of obstructive jaundice.

3) The reaction seems to be of value in the differential diagnosis between hepatitis and obstructive icterus.

4) The thymol test was made on 415 patients with various internal diseases. Positive reaction was observed in 36, *i. e.* about 9 %.

5) An examination of the relation between the thymol reaction and the icterus index shows when the findings in different cases are compared that there is no direct quantitative correlation, but a certain degree of chronological correlation (individually).

6) In 23 of 33 cases of acute hepatitis the thymol reaction remained positive at a time when the icterus index value had become normal.

7) An after-examination of patients discharged as presumably recovered but with an increased thymol value, revealed in 5 out of 12 cases relapses and re-increased thymol values.

Literature.

1. MacLagan, N. F.: »Nature» 154: 670: 1944. — 2. MacLagan, N. F.: »British Journ. of Exp. Path.» 25: 234: 1944. — 3. Recant, L., Chargaff, E. & Hanger, F. M.: »Proceed. of the Soc. for Exp. Biol. and Med.» 60: 245: 1945. — 4. Brante, Gunnar: »Svenska Läkartidningen» 43: 2661: 1946. — 5. Cohen, Ph. P. & Thompson, F. L.: »Journ. of Lab. and Clin. Med.» 32, Nr. 5: 475: 1947. — 6. King, E. J. & Haslewood, G. A. D.: »The Lancet» Nov. 14: 1153: 1936. — 7. Kingsbury, F. B.: Clark, Ch. P., Williams, G. & Post, A.: »The Journ. of Lab. and Clin. Med.» 11: 981: 1926. — 8. Shank, R. E. & Hoagland, C. L.: »The Journ. of Biol. and Clin. Med.» 162: 133: 1946. — 9. Watson, C. J. & Rappaport, E. M.: »The Journ. of Lab. and Clin. Med.» 30: 910: 1945. — 10. Ley, A. B., Lewis, J. & Davidson, C.: »The Journ. of Lab. and Clin. Med.» 3: 910: 1946. — 11. Bing, H. I. & Heschker, H.: »Biochem. Zeitschr.» 149: 83: 1924. — 12. Blix, G.: »Acta Med. Scand.» 64: 142: 1926. — 13. Kunkel, H. G. & Hoagland, C. L.: »Proceed. of the Soc. for Exp. Biol. and Med.» 62: Nr 2: 1946. — 14. Thomsen, S.: Treatise. 1942. — 15. Bang, J. & Wanscher, O.: »Nord. Med.» 24: 2175: 1944. — 16. Martin: »British Journ. of Exp. Path.» 27: 363: 1946. — 17. MacLagan, N. F.: »The Biochem. Journ.» 39: XI: 1945. — 18. MacLagan, N. F.: »The Biochem. Journ.» 39: XXII: 1945.
-

From the I. Medical Clinic of the University of Budapest.
(Director: Prof. Stephen Ruzsnyák.)

The Effect of Thiamine (Vitamin B₁) on the Utilisation of Carbohydrates by the Tissues.

By

IMRE MAGYAR and PAUL RESOFSZKI.

(Submitted for publication October 6, 1947.)

One of the authors (1) found in 1938 that thiamine increases the effect of Insulin by easing the infiltration of it into the tissues. These investigations were strongly against the supposition that thiamine increases the insulin-production but did not rule out the possibility that the main factor is the rise of the peripheral sugar-utilisation.

The difference in the sugar content between the arterial (capillary) and venous blood (henceforth AV diff.) may be regarded as the measure of the peripheral utilisation of sugar (2, 3, 4).

The arterial (capillary) blood contains the whole quantity of sugar being at the disposal of the tissues, whereas the venous blood carries only the unused residual. The more sugar gets into the tissues, the more manifest will be the AV diff. In diabetes this difference decreases. Insulin increases this difference, which fact serves as a proof of its peripheral effect (2). The AV diff. depends of course on the speed of the blood-stream too (5).

Aim of our present work was to investigate the thiamine effect on the AV diff. after insulin and after administration of dextrose in healthy persons and in diabetic patients.

We know from our own work (1) and from the communications of others (6) that thiamine itself does not influence the blood sugar level in the fasting state. It seemed therefore plausible that it has no effect on the AV diff. Determination of the AV diff. 15, 30, 45 and 60 minutes respectively after administration of thiamine

confirmed this assumption. In the first part of our experiments we followed the behaviour of the AV diff. for one hour before the administration of the thiamine. We found that our method does not allow the evaluation of AV differences below 15 mg p.c., because under this limit spontaneous variations also occur. The determination of the AV diff. after thiamine did not show higher values than 15 mg p.c., indicating that thiamine itself had not influenced the AV diff.

Number	Name	Diagnosis	Fasting level	15	30	45	60	After 50 mg of thiamine				
								15	30	45	60	min.
1....	S. M.	neurasth.	92	81	76	78	80	76	84	90	95	cap.
			92	84	76	73	72	72	96	100	102	ven
2....	M. P.	neurasth.	100	91	97	92	88	90	100	101	100	cap.
			92	92	98	86	100	105	99	98	100	ven
3....	K. S.	gastritis	112	99	95	88	82	80	87	93	87	cap.
			106	92	99	99	90	92	84	83	80	ven

Later we investigated the thiamine effect on the AV diff. increased by the administration of dextrose or insulin. We employed the following method:

The investigation of the patient was carried out by both of us at the same time. One of us drew blood from the cubital vein not or slightly compressing it, whereas the other took blood from the punctured fingertip. After 12 hours of fasting the patient had his blood taken and got 30 gr dextrose dissolved in 200 ml of water, or 4, 6 or 8 units of insulin intravenously, according to his weight. Blood was taken again by the above described method, 15, 30, 60 and 90 minutes later. The blood sugar determinations we carried out with three, later with two samples employing the Fujita-Iwatake modification of the Hagedorn Jensen procedure (7). Two, respectively five days later the experiment was repeated on the same patient under exactly the same conditions, plus administering 50 mg Thiamine (Vitaplex B₂ Chinoin) into the cubital vein, with the dextrose or insulin respectively.

Our five dextrose experiments (No 4—8) one of them diabetes yielded very indistinct AV differences, the sugar content of the arterial and venous blood being very near to each other. The AV diff. did not rise after dextrose and thiamine either. We already knew from the investigations made earlier by one of us (1) that the curve of blood sugar after dextrose administration flattens and shortens through the thiamine effect. This was found in our pres-

ent investigations too. But contrary to Horn's (8) findings, we failed to demonstrate the thiamine effect on the AV diff. after dextrose.

Num- ber	Name	Diagnosis	Fasting 1	30 gr dex- trose			Fasting 1	30 gr dextrose + 50 mg thiamine			
				15	30	60		15	30	60	min.
4....	N. F.	pern. anaemia	91	117	119	128	94	108	113	122	cap.
			95	106	124	135	97	96	105	114	ven.
5....	L. M.	hyperthyreosis	78	90	146	133	78	101	130	120	cap.
			71	100	130	128	76	110	132	125	ven.
6....	M. K.	hyperthyreosis	108	160	193	174	104	155	182	152	cap.
			114	148	180	172	104	138	174	158	ven.
7....	S. A.	myodeg. cordis	83	122	166	166	86	108	127	145	cap.
			78	124	140	147	80	101	132	140	ven.
8....	T. S.	diabetes	142	187	223	228	136	173	213	227	cap.
			146	178	220	235	133	164	223	227	ven.

Thiamine made the AV diff. after dextrose rather decrease, and not at all increase. The fact that the AV diff. was very small after dextrose made the evaluation very uncertain.

Insulin caused a much more distinct AV diff. Even therefore the greatest part of our experiments was made after insulin. (In all 30 exp. 10 of which in diabetes.) Fig. 1 shows the planimetric evaluation of the blood-sugar numbers in the capillaries (arteries) and veins taken after insulin with thiamine respectively.

The area $ABCA_1$ represents the arterial (capillary), the area $VBCV_1$ the venous blood-sugar level, their difference shows planimetrically the AV diff. after insulin. On the right side chart we plotted the corresponding values taken after insulin plus thiamine. The change in the AV diff. caused by thiamine is the difference between the areas AVV_1A_1 and avv_1a_1 . If the thiamine would increase the peripheral utilisation of the blood sugar, the avv_1a_1 area should be larger than the area AVV_1A_1 i. e. the difference should be a positive number.

Before evaluation of our numbers we had to examine the effects of external circumstances or methodical errors. To this end we tested our method by carrying out one and the same experiment on one person within several days. (AV diff. after insulin.) Out of 5 experiments (No 9—13) the cases No 11 and 13 showed the greatest deviation.

IMRE MAGYAR AND PAUL RESOFSZKI.

Number	Name	Dagnosis	Fasting level	6 u. insulin				Fast.	6 u. insulin				
				15	30	45	60		15	30	45	60	min.
11..	P. H.	neurasth.	100 75	78 58	58 42	64 46	64 50	100 86	80 60	58 30	70 48	84 66	cap. ven.
13..	M. K.	malaria	72 78	75 63	58 48		60 52	92 78	75 55	56 32		100 78	cap. ven.

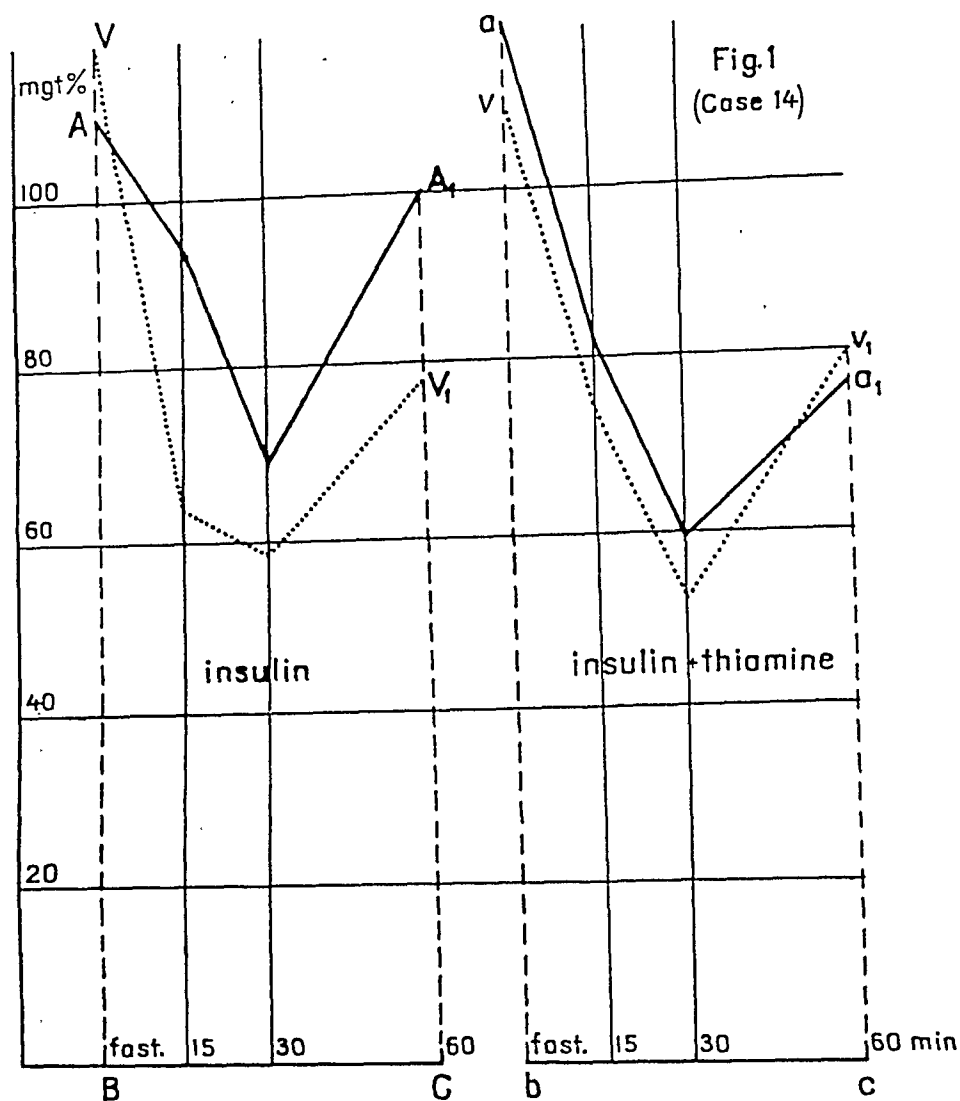
In these two cases the planimetric evaluations are the following:

Number	Art.	Ven.	AV diff.	Art.	Ven.	AV diff.	Change in	
							mm ²	%
11.....	2783	2072	711	2985	2120	865	+ 154	+ 22
13.....	2672	2242	430	3023	2189	834	+ 404	+ 91

Though the deviations of the other experiments are under 20 p.c., with regard of the 94 p.c. cast in exp. No 13, we did venture to evaluate difference under this figure.

The results of our experiments with insulin and insulin + thiamine are the following:

Number	Name	Diagnosis	Fast. I.	4-8 u. insulin			Fast.	4-8 u. insulin + 50 mg thiamine				
				15	30	60		15	30	60	min.	
14	K. A.	emaciation	109 110	94 63	67 58	99 78	119 110	83 74	59 52	77 80		cap. ven.
15	K. J.	neurasth.	99 100	88 83	82 78	98 85	99 99	65 72	51 45	86 80		cap. ven.
16	R. E.	st. p. infl.	96 91	63 59	68 71	87 85	85 83	58 42	58 52	60 60		cap. ven.
17	L. I.	hyperthyr.	120 113	92 84	78 78	100 85	100 100	92 88	87 63	77 66		cap. ven.
18	B. A.	obesity	102 100	74 70	76 63	98 72	102 109	64 48	55 50	91 55		cap. ven.
19	H. R.	sinusitis	87 84	66 62	62 59	78 77	97 98	64 46	57 44	67 49		cap. ven.
20	N. D.	gastritis	119 122	116 99	94 85	108 119	120 130	106 97	73 75	96 106		cap. ven.
21	T. M.	pleuritis	139 141	104 102	90 92	117 103	139 136	102 102	97 99	118 106		cap. ven.
22	N. V.	tbc	110 98	79 62	69 58	102 87	104 108	86 75	64 65	103 102		cap. ven.



Num- ber	Name	Diagnosis	Fast. l.	4-8 u. insulin			Fast.	4-8 u. insulin + 50 mg thiamine			
				15	30	60		15	30	60	min.
23	H. E.	hyperthyr.	98	70	59	71	88	47	34	47	cap.
			83	50	53	64	76	39	31	36	ven.
24	G. R.	emaciation	108	78	68	97	111	83	60	67	cap.
			103	97	70	80	105	91	70	62	ven.
25	K. J.	hypertension	123	102	100	118	110	72	77	86	cap.
			126	95	74	101	120	74	52	77	ven.
26	Sz. E.	polyarthriti s acromeg.	88	77	72	74	84	56	67	78	cap.
			113	60	70	92	94	60	84	84	ven.

Num-ber	Name	Diagnosis	Fast. l.	4-8 u. insulin			Fast.	4-8 u. insulin + 50 mg thiamine			
				15	30	60		15	30	60	min.
27	K. E.	duod. ulcer, emaciation	104 110	110 103	91 95	62 71	118 114	66 62	58 52	76 76	cap. ven.
28	D. S.	nephrolith.	96 103	76 73	52 40	62 68	94 100	72 64	38 43	70 71	cap. ven.
29	V. J.	neurasth.	108 88	86 53	55 57	72 64	112 103	60 58	58 64	76 83	cap. ven.
30	M. J.	arterioscl.	133 128	108 94	100 63	70 54	133 137	101 108	83 88	76 72	cap. ven.
31	B. Gy.	colitis	102 110	90 83	63 53	65 63	95 102	83 67	54 58	68 75	cap. ven.
32	B. J.	coxitis	117 110	115 87	90 83	102 99	112 105	88 81	72 62	96 74	cap. ven.
33	T. S.	arthr. def.	95 101	92 80	82 70	88 76	98 100	82 78	64 63	72 69	cap. ven.
Diabetic cases:											
age:											
34	B. M.	50	186 192	160 166	171 156	174 166	180 170	158 153	123 122	92 92	cap. ven.
35	H. L.	50	150 146	102 85	83 74	97 108	180 178	126 96	96 81	118 88	cap. ven.
36	D. J.	62	221 230	193 198	144 151	119 109	226 220	198 173	135 136	98 90	cap. ven.
37	G. I.	54	151 144	133 128	96 100	76 56	153 145	113 98	86 70	70 59	cap. ven.
38	R. R.	64	163 163	141 122	116 113	114 91	161 165	132 111	111 102	91 83	cap. ven.
39	D. L.	68	186 198	180 169	176 165	151 100	190 180	162 153	116 117	108 95	cap. ven.
40	H. Zs	20	218 222	183 177	169 154	152 138	227 225	187 187	144 132	122 124	cap. ven.
41	M. J.	51	197 218	174 160	158 116	131 91	172 195	165 147	123 96	85 68	cap. ven.
42	K. Gy.	65	240 260	246 220	202 188	185 156	258 258	217 191	203 189	151 148	cap. ven.
43	Sch. F.	62	258 262	260 258	258 208	244 222	256 254	244 245	222 181	184 178	cap. ven.

The following table contains the planimetric values and their results. The values are arranged in the order of the percentual change of the AV diff. The AV diff. after insulin and the AV diff. after insulin + thiamine are given and further their difference in mm² and p.c. The increase of the AV diff. is indicated with +, the decrease with -. A separate column contains the thiamine

effect on the blood-sugar level after insulin in planimetric values, which represents a repetition and confirmation of the experiments of 1938 (1). The diabetic cases are printed in italics.

No	Num- ber of case	Insulin			Insulin + thia- mine			Effect of thiamine		Change of the AV. diff. in	
		Art.	Ven.	AV. diff.	Art.	Ven.	AV. diff.	Art.	Ven.	mm ²	p.c.
1	36	6338	6451	- 113	5966	5743	223	- 372	- 708	+336	+297
2	27	3554	3713	- 155	2846	2668	178	- 708	-1045	+337	+220
3	19	2802	2687	115	2441	2093	348	- 361	- 594	+233	+203
4	37	4272	4049	223	3849	3333	516	- 518	- 716	+293	+132
5	17	3677	3412	265	3568	2969	599	- 109	- 443	+334	+126
6	16	2996	2837	159	2470	2195	275	- 526	- 642	+116	+ 73
7	34	6723	6614	109	5255	5125	134	-1468	-1493	+ 25	+ 23
8	18	3368	2859	509	2880	2316	564	- 488	- 543	+ 55	+ 11
9	21	4250	4134	116	4333	4217	116	- 83	- 83	-	-
10	32	4203	3643	560	3495	3000	495	- 708	- 643	- 65	- 12
11	38	5093	4636	457	4689	4302	387	- 404	- 334	- 70	- 15
12	42	8471	7884	587	8016	7532	484	- 455	- 352	-103	- 18
13	15	3566	2754	812	3346	2689	657	- 220	- 65	-155	- 19
14	23	2772	2360	412	1881	1566	315	- 891	- 794	- 97	- 24
15	42	10190	9213	977	8705	8044	661	-1485	-1169	-316	- 32
16	25	4306	3677	629	3261	2855	406	-1045	- 822	-223	- 35
17	41	6404	5291	1113	5181	4611	570	-1223	- 680	-543	- 49
18	35	4659	3926	733	3961	3670	291	- 698	- 356	-442	- 50
19	14	3467	2887	580	3071	2849	222	- 396	- 38	-358	- 62
20	39	6895	6143	752	5402	5123	279	-1493	-1020	-473	- 63
21	40	7083	6572	511	6355	6170	185	- 728	- 402	-326	- 64
22	22	3385	2835	550	3351	3241	110	- 34	+ 406	-440	- 80
23	33	3506	3122	384	2966	2912	54	- 540	- 210	-330	- 86
24	31	2995	2783	212	2788	2789	- 1	- 207	+ 6	-213	-100
25	30	3930	3030	900	3676	3798	-122	- 254	- 768	1022	-114
26	29	2738	2459	279	2757	2881	-124	+ 19	+ 422	-403	-145
27	20	4236	4054	182	3711	3806	- 95	- 525	- 248	-277	-152
28	28	2633	2483	150	2301	2466	-165	- 332	- 17	-315	-210
29	26	3013	3118	- 105	2740	3159	-419	- 273	+ 41	-314	-299
30	14	3303	3327	- 24	2924	3109	-185	- 379	- 218	-161	-671

The data of the table show that except of 4 cases (No 1, 2, 29, 30) a distinct AV diff. emerges after insulin. Administration of thiamine in 30 cases caused decrease in 21, increase in 8 cases, leaving the AV diff. unchanged in 1 case. If we exclude the deviations under 100 p.c. corresponding to the cast of the control experiments, it can be stated that the AV diff. after insulin was increased in 5 cases, decreased in 6 cases and left unchanged in 19 cases by thiamine.

As the table shows, out of 30 cases the arterial blood sugar level was decreased in 29 cases, the venous blood sugar in 24 cases by thiamine. The decrease of the sugar content of the venous blood

means the augmentation of the sugar-utilization of the tissues. This finding is in accordance with the experimental results of one of us (1) demonstrating that thiamine facilitates the diffusion of insulin into the cells. As the AV diff. did not vary, we suppose that thiamine has not only a peripheral but a central, hepatic effect too, viz. it increases the glycogen-apposition-effect of the insulin. The mechanism of this effect seems to be similar to the peripheral one; thiamine increases the diffusion of insulin into the cells. On account of the balance of the peripheral and central (hepatic) effect, the AV diff. after insulin did not change in the most cases. The greater AV diff. in some cases means the preponderance of the peripheral effect, a lower one the greater intensity of the liver-effect. The closer inspection of the cases showed that the greatest decrease of the AV diff. occurred with Simmonds disease and acromegalia (cases 26, 14). We found in these two patients that their arterial blood-sugar level was consequently lower than the venous one, thus an AV diff. did not appear even after insulin. Likewise behaved the two other extreme cases of diabetes and pituitary emaciation (Cases 36, 27). It seems that endocrine glands, at first the hypophysis influences the effect of thiamine. From the investigations of Julesz (9) we know that thiamine hinders the activity of the frontal lobe of the hypophysis. Julesz explains the thiamine-effect on the carbohydrate metabolism with the inhibitive impact of thiamine on the frontal lobe of the hypophysis. Contrary to Julesz, we think, that the hypophysis only influences the effect of thiamine on insulin, on the same way as it influences the effect of insulin.

From the point of view of carbohydrate metabolism there is no essential difference between the behaviour of healthy and diabetic persons. Our table shows, however, the diabetic cases among those ones in which the thiamine effect either increases the AV diff. or lets it unchanged. No decrease of the AV diff. was registered in diabetes by thiamine.

Summary.

After administration of dextrose and insulin we determined the difference of the blood sugar content in the arterial and venous blood, *i. e.* the extent of the sugar utilisation of the tissues, and examined the effect of thiamine on this difference. We found that thiamine fails to change the AV diff. We complete our earlier fin-

dings, concerning the fact that thiamine facilitates the diffusion of insulin into the cells, with the statement that this effect acts not only on the periphery, but centrally, on the liver, too. The balance of the central and peripheral effect of the thiamine depends probably on the activity of the hypophysis.

We are expressing our thank to the chemical factory Chinoin RT Budapest for the generously given material (Vitaplex B₁ Chinoin) and to Miss Susan Abelsberg, laboratory assistant, for her valuable help.

References.

1. Magyar I.: Zeitschrift f. d. ges. exp. Med. 104. 495. 1938. — 2. Cantarow, A. and Trumper, M.: Clinical Biochemistry. Saunders 1946. — 3. Foster, G. L.: J. Biol. Chem. 55. 291. 1923. — 4. Soskin, S. and Levine, R.: Carbohydrate Metabolism. The university of Chicago Press. Chicago 1946. — 5. Dumke, P. R. and Schmidt, C. F.: Am. J. of Physiol. 138. 421. 1943. — 6. Schröder, H.: Zeitschr. f. d. ges. exp. Med. 101. 402. 1937. — 7. Horn, Z.: Zeitschr. f. d. ges. exp. Med. 108. 411. 1940. — 8. Fujita Akiya and Danzo, Iwatake: Biochem. Zschr. 242. 43. 1932. — 9. Julesz, M.: Magyar Belorvosi Arch. 1. 83. 1947.
-

Publications Received.

- The Rockefeller Foundation.* A review for 1947, by Raymond B. Fosdick. New York, 1948.
- A Symposium on the Use of Isotopes in Biology and Medicine.* 445 p. Price: \$ 5.00. The University of Wisconsin Press, Madison, Wisconsin, 1948.
- Edmond Sergent et Etienne Sergent:* Histoire d'un Marais algérien. 293 p. Institut Pasteur d'Algérie, Alger, 1947.
- Antonio Battro:* Las arritmias en clinica. Diagnóstico, pronóstico y tratamiento. 511 p. 299 fig. Editor »El Ateneo«, Buenos Aires, 1948.
- Ib Fabricius Hansen:* Investigations on agonal acidosis. 134 p. Stechert-Hafner, Inc., New York, and Poul Branner, Copenhagen, 1948.

From the Medical Department B of the Rigshospital, Copenhagen.
(Chief: Professor Erik Warburg, M. D.)

Chronic Constrictive Pericarditis.

By

VAGN MORTENSEN and ERIK WARBURG.

(Submitted for publication October 22, 1947.)

Introduction.

During the past 24 years, since Volhard & Schmieden published their epoch-making paper on the diagnosis and treatment of this syndrome, constrictive pericarditis has attracted increasing attention. As a matter of fact this syndrome was no new observation, insofar as Lower has described it as early as in 1669, saying (cited after P. D. White):

»Although the fluid enclosed in the pericardium serves effectively for lubricating the surface of the heart and facilitating its movement, it sometimes happens that a profuse effusion oppresses and inundates the heart. This envelope becomes filled in hydrops of the heart; the walls of the heart are compressed by the fluid settling everywhere so that they cannot dilate sufficiently to receive blood; then the pulse becomes exceedingly small, until finally it becomes utterly suppressed by the great inundation of fluid, whence succeed syncope and death itself . . . Just as the accumulation of too much water in itself brings harm to the heart so it happens that trouble comes when heart and pericardium become everywhere closely adherent; whence, when it becomes attached also to the diaphragm the motion of the heart necessarily mingles and combines with that of the diaphragm.»

In France, towards the end of the past century, Hutinel called attention to the connection between symphysis of the peri-

cardium and portal stasis — indeed, the disease is often named after Hutinel. Finally, in their theses, Boissin, Boutavant and Cousin have given a thorough description of the pathological-anatomical aspects of the lesion. These were often misinterpreted and at that time the French school thought — as far as we can see, erroneously — that the chronic constrictive pericarditis usually was of tuberculous etiology.

The paper published by Friedel Pick, from Pribran's Clinic in Prague, meant a considerable progress in the conception of the syndrome, as he understood the mechanism of the stasis here involved *almost* completely.

In Denmark, in 1902, Mohr gave an excellent survey of the syndrome, which here was of particular interest through the circumstance that our famous fellow-countryman Niels Finsen, the father of the medical light-therapy, died of this lesion.

Since the operative treatment of this syndrome has become of great significance — through the work of Brauer and, especially Delorme — the interest in the disease has increased markedly. The operative treatment turned out successful for the first time in 1913, in which year Sauerbruch and Rehn each operated on a case of this kind. The operative treatment was taken up by Schmieden in 1918, who since has operated on a large number of cases — up to 1944 a total of 54 cases, undoubtedly the largest number recorded for a single surgeon — and who must be said to have been the leading surgeon in this field through a number of years.

Outside Germany, particular interest in this syndrome has been taken in U. S. A. and in the Scandinavian countries. In U. S. A. the first case was treated operatively in 1929 by Churchill. Since then, in particular, 3 medical centers have been dealing with this syndrome, namely: in Cleveland, Ohio (Beck), in Boston (White and Churchill), and in Nashville, Tennessee (Burwell, Blalock and Strayhorn), from which series of operated cases have been reported, Beck having nearly the same number of cases as Schmieden. More recently the operation has been performed in all fairly large medical centers in U. S. A. Among others, in 1944, altogether 24 cases were reported from the Mayo Clinic by Harrington, and 18 cases from New York by Heuer & Andrus.

In the Scandinavian countries, especially Ingvar (in Sweden) and Bøggild and Warburg (in Denmark) were early interested

Significance of Persisting Positive Thymol Reaction after Apparent Clinical Restitution.

Kunkel & Hoagland (13) applied the thymol test in convalescence after acute hepatitis and found it to be positive in 67 % of 27 cases with persisting symptoms (the nature of which is not stated). Of 30 without symptoms only 7 % had a high thymol value.

About 18 % of the cases of acute hepatitis examined at the Hospital of the Rockefeller Institute, N. Y. had a clinical relapse during convalescence. In all these cases there was an increase in the thymol value one to three weeks after the relapse set in. In some instances the thymol value was higher than at the primary attack, and in some the increased values persisted up to six months after other objective signs of the relapse had subsided.

For the purpose of examining the significance of a persistently high thymol value, 12 of the patients discharged as apparently recovered, while still having a high thymol value, were examined subsequently (fig. 4).

Special interest attaches to the curves of five of the patients because, in contrast to the others who were after-examined, they still presented both subjective (lassitude, nausea, vomiting) and objective symptoms (jaundice [sub-jaundice]) of defective restitution.

As these cases also seem to be of particular interest in the question of the significance of a persistently positive thymol reaction, their data are given below in brief outline (Th. = thymol value, TA. = Takata Ara, II = icterus index).

No. 1 (♀ b. 14/12-20) (Curve 1) on discharge had Th. = 0.27, TA. negative, II. 8. The after-examination shows considerable lassitude, occasional epigastric oppression and nausea, but no jaundice. Four weeks after discharge, Th. was 0.67, TA. positive.

No. 2 (♀ b. 1/5-13) on discharge had Th. 0.42, TA. weakly positive, II. 7. Constant lassitude since discharge. In the period three to five weeks after discharge had a temperature, about 38°, sometimes nausea and epigastric oppression. At this juncture there is marked fluctuation in Th. to 0.75. TA. as on discharge, weakly positive, II. 12. Fourteen days later a subjective improvement, temperature normal, and a considerable fall in Th. to 0.35. TA. still weakly positive, II. only 6.

No. 3 (♀ b. 24/3-24) on discharge had Th. 0.17, TA. negative, II. 5, but in the ten weeks prior to the after-examination had two relapses with the symptoms lassitude, nausea, vomiting, pale faeces, dark urine. At the after-examination the only complaint is tiredness. Th.

in the syndrome and have published series of operated cases in 1937—1939, the operations being performed respectively by Tengvall, Boggild, Lendorf and Kjærgaard. The 9 cases previously published by Warburg are included in the patient material here presented.

In France, pericardiectomy was performed by Hallopeau as early as 1910. After this, however, the operative treatment of the syndrome appears not to have attracted any particular interest until 1939, when the first operated cases with non-fatal outcome were reported by Santy, Bernheim, Piquet & Galy. In the same year, a thesis was published by Piquet on the syndrome, comprising 59 operated cases gathered from the literature.

In the case of England the same holds true as has been said about France: that some very valuable contributions to the clinical aspects of the syndrome were given at an early juncture, whereas the operative treatment appears to have been slow in attracting a similar interest as it met with in Germany, U. S. A. and the Scandinavian countries.

Now the total number of operated cases amounts to several hundreds. In 1936 the total number of operated cases was reckoned to be 110 (an account given by Smith & Liggett in 1928 comprises the total of 107 cases, but undoubtedly many of these cases have not been clear-cut — or several of them may have been diagnosed erroneously — as valvular defects were present in no less than 29 cases). Since then, the number of operated cases has been increasing markedly. In 1944 the two largest series of cases reported from a single clinic had grown to about half a hundred cases, and in addition numerous smaller series and single observations have been published.

In the literature it is generally agreed that patients suffering from chronic constrictive pericarditis should be treated operatively, but such treatment should be postponed as long as the patient shows any evidence of active infection. Still, Burwell & Blalock think that no particular risk is involved in operation for tuberculous constriction in the presence of active proliferative tuberculous pericarditis — a view that is not shared by Churchill. On the other hand, the operation should not be postponed too long, as the operative risk increases with increasing cardiac insufficiency, especially when the process of calcification appears in the pericardium. Calcification is no contraindication for the operation, however, and, among others, Settergren advocates a greater liber-

literature concerning this point. Westermann, from Schmieden's clinic, is an ardent advocate of early operative treatment.

In the various statistics the operative results are fairly alike, with a case mortality of above 25 % in connection with the operation, or in the first years after, and 50—75 % cases of recovery or considerable improvement. Thus in Schmieden's 54 cases, in 1914 Westermann found: 36.9 % recovered, 28.3 % considerably improved, 15.1 % died during, or shortly after, the operation, 5.7 % died in the period of after-treatment, and 15 % died later after transitory improvement. Of 46 patients operated on by Beck, 67.4 % recovered, 4.3 % improved, 2.2 % died under the operation, 17.4 % died in the postoperative period, and 8.7 % died later on. These statistics are essentially alike, as to a large extent it is a matter of definition who are to be designated as recovered or considerably improved. Recovery is defined as a state of health in which the patient is able without any particular trouble to resume his ordinary occupation.

Concerning the literature on chronic constrictive pericarditis, further mention is to be made only of various views as to the etiology of the lesion. In the course of time these views have been changing a good deal, and the question is not settled yet. At an early juncture the French school took the etiology to be tuberculous. Later on rheumatic infection was taken to be responsible for the constriction of the heart in most of the cases. Thus, in 46 % of their 107 cases gathered from the literature, Smith & Liggett found the history of rheumatic infection. But, as mentioned before, the material collected by these authors includes so many instances of valvular defects that it hardly may be looked upon as clear-cut. Tuberculosis is mentioned as the second most frequent cause (29 %).

The more recent works fail to confirm that rheumatic infection is particularly frequent in constriction of the heart (White, Warburg). The earlier view of rheumatic infection being particularly frequent in this lesion is due to the circumstance that no sufficiently sharp distinction has been made between pericarditis in general (which most often is of rheumatic origin) and the special form of chronic pericarditis that gives the constriction of the heart; to the latter a rheumatic infection is of no etiological significance. King even goes so far as to state that a history of rheumatic infection should make us sceptic about the diagnosis of constriction of the heart.

Table 2.
Age Distribution of the Patient Material.

	0—10 years	11—20	21—25	26—30	31—40	41—50	51—60	61—70
Age at onset of symptoms	1	7	6	2	4	2	2	1
Age at the time of diagnosis	0	6	6	3	4	3	2	1

It will be noticed that constriction of the heart preferably attacks the young age-classes, 14 of the patients presenting symptoms of this lesion before the age of 25 — one-third of the patients even before the age of 20 years. In our material no difference can be demonstrated in the age distribution of male and female patients.

Admitting Diagnoses.

Altogether 14 patients were admitted for chronic constrictive pericarditis, observation for this syndrome, or accretio cordis. All these patients, however, were transferred to our department from other special medical clinics. The remaining 11 cases were admitted under other diagnoses (*e. g.*, polyserositis, chronic hepatitis, or cardiac disease). It looks as if the general practitioners still have difficulties in making the diagnosis.

Etiology.

In the past histories of the patients we have looked into the occurrence of the diseases which from experiences are known not infrequently to give cardiac disease, besides the occurrence of tuberculosis and traumatic injury. The outcome is recorded in Table 3.

From Table 3 it will be seen that the diseases which not infrequently give some heart lesion here are represented but scantily. It is to be pointed out in particular that rheumatic fever occurred in the past history of only 2 patients, while 21 stated explicitly that they had never been suffering from rheumatic fever. Thus, the present material does not indicate that the rheumatic in-

Table 3.

Incidence of Diseases which not infrequently give Heart Lesions, besides the Occurrence of Tuberculosis and Traumatic Injury in the Past Histories of the patients.

	Rheumatic fever	Chorea	Scarlet fever	Diphtheria	Recurrent angina	Lymphadenitis	Pleurisy	Pulmonary tuberculosis	Other tuberculous manifestations	Traumatic injury
Present in	2	0	2	2	1	1	9	5	1	3
Absent in	21	7	15	17	4	4	1	1	1	0
No data	2	18	8	6	20	20	15	19	23	22

fection plays any particular rôle in the occurrence of constriction of the heart. The cases implying a possibility of a rheumatic etiology were as follows: 1 patient had perhaps had pericarditis after an attack of scarlet fever at the age of 8 years, but the constriction gave no symptoms till the patient had reached the age of 43. 1 patient gave a past history of rheumatic fever at the age of 18 years, and the constriction commenced at the age of 27. 1 patient said that perhaps he had had rheumatic fever and rheumatic pleurisy at the age of 16 years; in his case the constriction of the heart commenced at the age of 25. Finally, 1 patient showed an antistreptolysin titer of 1,135—800 on admission for constriction of the heart; 9 years before, the same patient had been suffering from pulmonary tuberculosis. It is not possible from these cases to say whether the past rheumatic infection may have been of any etiological significance to the subsequent constriction of the heart, or whether it merely is a matter of accidental coincidence. The frequency of rheumatic infection in the past history of the patients is no greater in the constriction material than in the rest of the patient material in this department, being about 10 %.

Among other etiological factors, mention is to be made of tuberculosis. The frequency of pleurisy in the histories of the patients offers no information, as possibly the «pleurisy» may merely have been a state of hydrothorax. In 3 cases the constrictive pericarditis was positively tuberculous (verified histologically). The group of uncertain and complicated cases further includes 2 cases of tuberculous constrictive pericarditis, both

complicated by tuberculous empyema. Thus there can be no doubt that tuberculosis may give rise to constriction of the heart. Possibly tuberculosis may have played an etiological rôle in many of the cases where no definite etiological factor could be found, but this is quite uncertain.

In 2 cases the etiology consisted in traumatic injuries, respectively blunt violence and gunshot injury. Finally, in 1 case the constriction of the heart was due to calcification of the pericardium following coronary occlusion.

In the remaining cases the etiology was quite obscure. In 7 cases the patients stated they had had an attack of a febrile disease, most often influenza, bronchitis or pneumonia — about at the time when the symptoms of the constriction commenced; and in the other cases no information could be obtained on this point.

So our knowledge concerning the etiology may be summarized to this effect: that it is doubtful whether the rheumatic infection plays any particular rôle in this respect, while tuberculosis may give rise to constriction of the heart, and the same applies to coronary occlusion and traumatic injury. On the whole, however, we know but very little about the etiology.

Complaints.

Table 4 gives a survey of the frequency of various complaints or symptoms observed by the patient himself.

Table 4.

Frequency of various Symptoms observed by the Patients themselves.

	Dyspnea	Increasing abdominal circumference	Edema	Cardiac pain	Palpitation	Dyspepsia	Cough	Dizziness
Present in	25	22	19	8	10	4	12	4
Absent in	0	3	6	12	11	12	10	0
No data	0	0	0	5	4	9	3	21

In addition, most of the patients also complained of tiredness. Thus the principal symptoms are dyspnea, increasing circum-

ference of the abdomen and tendency to edema, whereas cardiac sensations are less frequent. In nearly all the cases the initial symptoms were dyspnea, abdominal tension and increasing circumference of the abdomen, whereas edema of the lower extremities did not appear till later on.

Clinical Signs.

In Table 5 the frequency of various clinical signs is recorded. Thus, functional dyspnea and enlargement of the liver were found in all the 25 patients; and also the frequency of swollen

Table 5.

Frequency of various Clinical Signs of the Syndrome.

	Albuminuria	Pulmonary stasis. No hydrothorax	Hydrothorax	Edema	Ascites	Enlargement of the liver	Swelling of cervical veins	Cyanosis	Functional dyspnea	Resting dyspnea
Present in	4	2	14	16	20	25	21	18	25	11
Absent in	21	9	11	9	5	0	3	7	0	13
No data	0	0	0	0	0	0	1	0	0	1

cervical veins and ascites was very high. The appearance of these patients is so characteristic that often the diagnosis can be made merely on their looks — at any rate in the relatively young cases (see Figs. 1 and 2).

The more important auscultatory findings are given in Table 6.

Table 6.

The More Important Auscultatory Findings.

	Broadbent's inverted sign	Systolic retraction of the ietus	Ietus impalpable	Fixation of ietus	Paradoxical pulse	Accentuation of P ₂	Rough systolic murmur	Soft systolic murmur	Mesodiastolic gallop	Presystolic gallop	Heart sounds clear
Present in	0	8	11	17	1	11	1	1	16	1	6
Absent in	5	8	12	1	9	14	24	24	9	24	19
No data	20	9	2	7	15	0	0	0	0	0	0

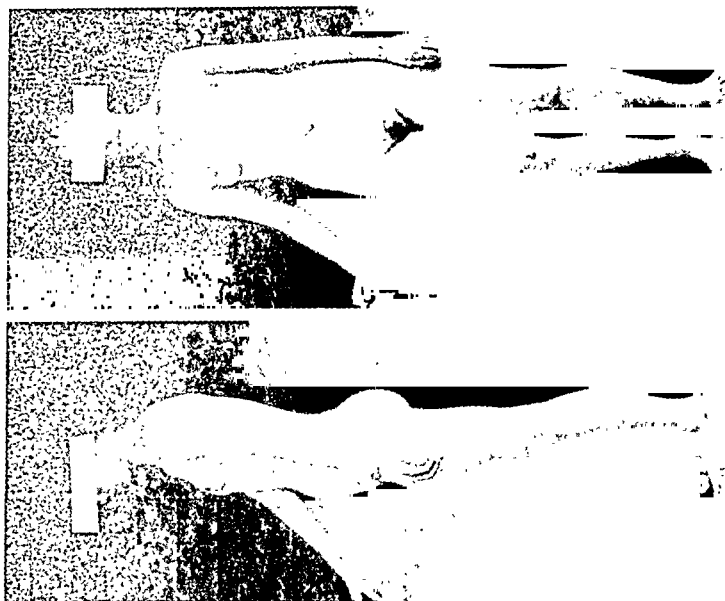


Fig. 2. Patient with constriction of the heart, after the operation.



Fig. 1. Patient with constriction of the heart, before the operation.

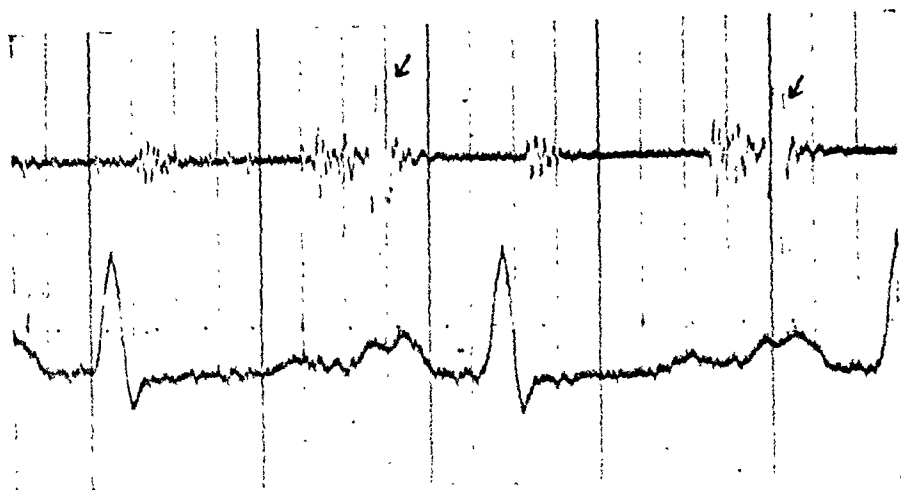


Fig. 3. Phonocardiogram of the pericardial click.

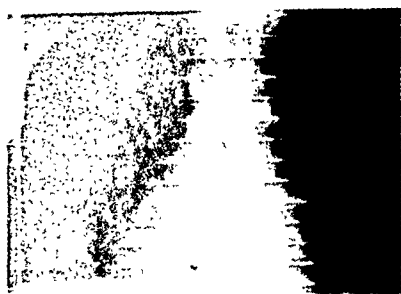
Unfortunately, no statement was made in the case records about several symptoms.

A particularly interesting feature in Table 6 is the very frequent occurrence of protodiastolic galop rhythm (*«la clique pericardiale»*). This click is of real diagnostic value. In contrast hereto we are rather sceptical about the fixation of the heart on changes in posture. At any rate the latter phenomenon is rather difficult to establish, whereas the pericardial click most often is easy to hear. Apart from this, only in one case was a murmur of any significance heard.

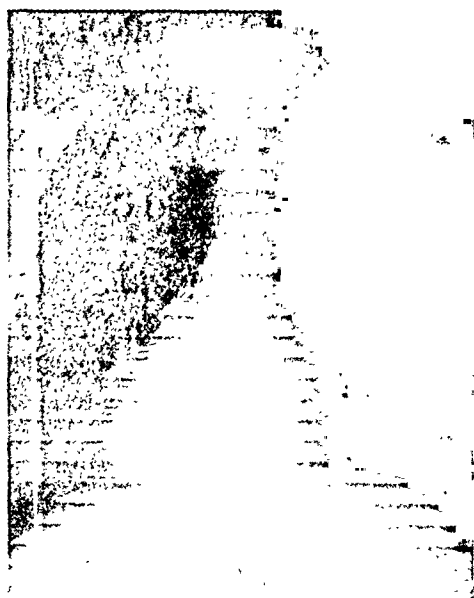
A phonocardiogram of the pericardial click is shown in Fig. 3.

X-ray Examination.

On *roentgenography* the heart was found to be of normal size in 18 cases, slightly enlarged in 2 and considerably enlarged in 5 cases. In one of the last-mentioned cases, however, the enlargement was due to a voluminous pericardial exudate, while the constriction of the heart was produced merely by a greatly thickened visceral pericardium, and the heart was normal in size. No characteristic change in the form of the heart was found in this material. In 12 patients, areas of calcification could be demonstrated in the pericardium; and this applies to 5 of the 6 cases in which the heart actually was enlarged. All the patients showing pericardial calcification were men. Still, in one additional case an insignificant degree of pericardial calcification appeared in a

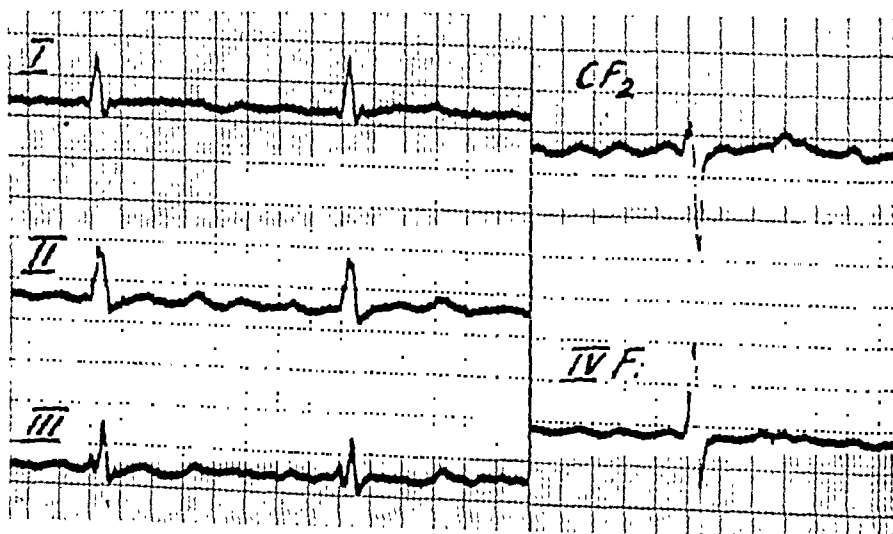
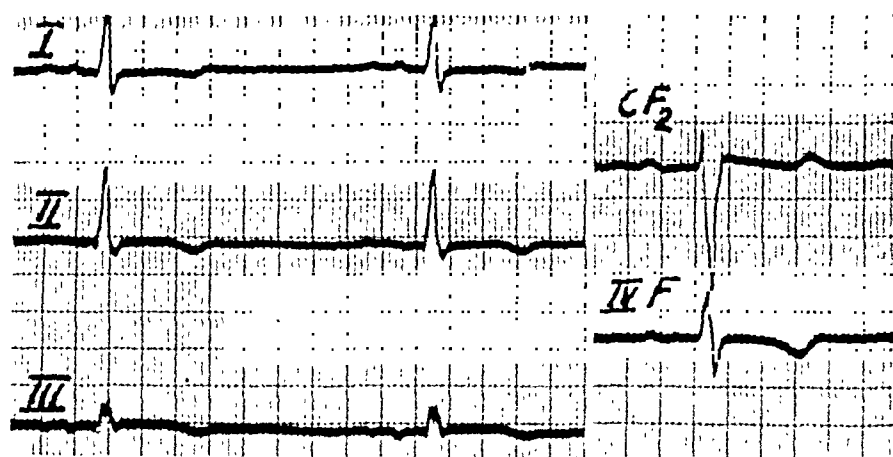
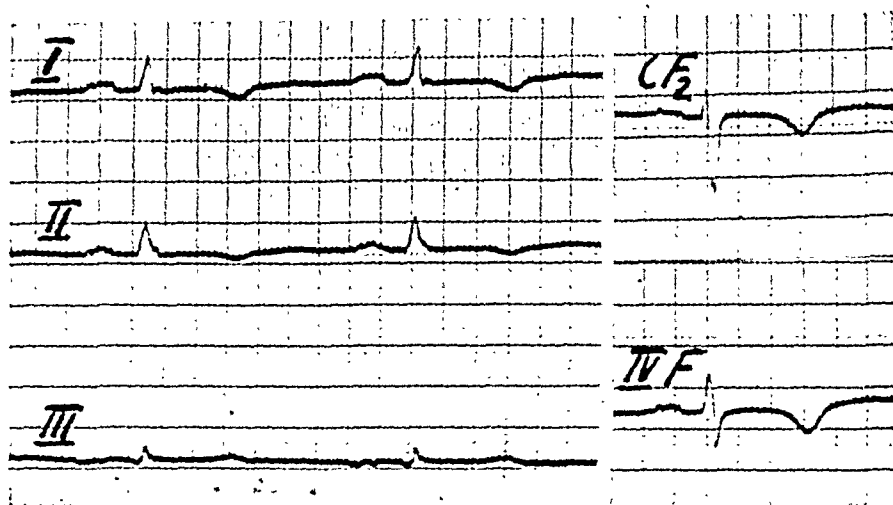


a



b

Fig. 4. Kymogram before and after the operation.



Figs. 5, 6 and 7. Different types of electrocardiograms in chronic constrictive pericarditis.

woman several years after the operation. As will be mentioned below, 5 patients had arrhythmia perpetua, and they all showed pericardial calcification. Thus, enlargement of the heart, areas of calcification in the pericardium and the arrhythmia perpetua have a tendency to accompany each other.

Kymography was performed in 18 cases and showed invariably small deflections or none at all. In 7 cases, kymography was performed after the operation; and in 3 of these cases there was no change in the picture, while in 2 cases the deflections have become larger, and in 2 cases the kymogram had become normal.

The kymogram obtained in one of our cases before and after the operation is shown in Fig. 4.

Electrocardiography.

The most frequent electrocardiographic changes consist in inversion of T waves. The following changes were found:

— T_1 , — T_2 and — T_3 : 9 cases.

Diphasic or iso-electric T_1 , — T_2 and — T_3 : 8 cases.

+ T_1 , — T_2 and — T_3 : 4 cases.

Iso-electric or negative T_1 and T_2 ; negative, positive or iso-electric T_3 : 4 cases.

Precordial leads (parasternal and apical) were employed in 22 cases, 9 of which showed inversion of the T wave in both leads, while 6 showed inversion of the T wave in the apical lead, and the remaining cases showed positive T waves in both precordial leads.

Left ventricular preponderance did not occur. Right preponderance was found in 3 cases. Low voltage was found in 7 cases. Arrhythmia perpetua was found in 5 cases; one of these patients showed first auricular fibrillation and later, after the operation, auricular flutter, which on treatment with quinidine was replaced by sinus rhythm. Various types of electrocardiograms are shown in Figs. 5, 6 and 7.

Venous Pressure.

The height of the venous pressure in these cases is recorded in Table 7.

The venous pressure is greatly increased in all the cases examined but one, and here it was increased in the femoral vein.

Table 7.
Venous Pressure.

	Not measured	130	151—200	201—250	251—300	301—351	400
Venous pressure in Nos. of patients	2	1	2	5	8	5	2

From our material as well as from nearly all others published, then, it is evident that measuring of the venous pressure is of the very greatest importance to clinching of the diagnosis.

Arterial Pressure.

The arterial blood pressure is of minor importance in cases of this kind. Our findings are recorded in Table 8.

Table 8.
Arterial Blood Pressure.

	80	90	100	110	120	130	140	150	210
Systolic pressure, maximal in Nos. of cases	1	1	2	8	5	5	1	1	1
	50	60	70	80	90	100	110	120	
Diastolic pressure, maximal in Nos. of cases	1	4	6	7	5	0	1	1	

A relatively low systolic pressure is a characteristic feature. The one instance of marked hypertension was found in a man, 65 years old, with a past history of coronary thrombosis.

It has been claimed that a low blood pressure amplitude is particularly characteristic of constriction of the heart. On this account the blood pressure amplitudes are recorded in Tables 9 and 10, from which it will be noticed that even though the amplitude often is low, this is no obligate symptom.

Operative Results and Indications for Operative Treatment.

Of the 25 patients 20 were given operative treatment (Delorme's operation). As in several of these cases the operation was performed

in the last years, the observation period for some patients naturally has been rather short. Still, the material gives some valuable information about the operative results. In Tables 9 and 10 all the cases are entered with various data that are of interest in judging of the operative results.

Table 9.
Various Data on Patients given Operative Treatment.

	Case No.	Age at operation	Pericardial calcification	Arrhythmia perpetua	Hypertrophy of the heart	Etiology	Duration of illness before operation	Duration of illness after operation	Total duration of illness	Pulse pressure
Group I Dead	4	15	0	0	0	?	2 years	4 years	6 years	30-95
	11	17	0	0	0	?	2 "	4 days	2 "	40-40
	28	19	0	0	0	?	8 mths.	<1 day	8 mths.	35-60
	14	22	+	0	+	?	18 "	6 weeks	18 "	30-45
	10	24	+	0	0	trauma	8 years	3 days	8 years	40-60
	1	25	0	0	0	?	2 "	6 years	8 "	20-55
	27	27	+	0	0	?	2 "	0	2 "	40-45
	30	34	0	0	0	tuberculosis	2 "	2 mths.	26 mths.	20-50
	33	34	0	0	0	"	2 "	2 days	2 years	20-30
	29	46	+	+	0	?	18 mths.	2 days	18 mths.	25-40
Group II Unchanged	22	51	+	0	0	?	6 years	2 hours	6 years	20-30
	24	24	+	0	0	?	1 year	>2 mths.	>14mths.	30-40
Group III Greatly improved	15	27	+	+	+	?	7 weeks	>7 mths.	>9 mths.	30-40
	5	14	0	0	0	?	3½ years	>6 years	>9½ yrs	30-45
	3	15	0	0	0	?	2 "	>10 "	>12 "	20-20
	12	22	0	0	+	?	6½ "	>5½ "	>12 "	30-40
	2	25	0	0	0	?	4½ "	>8 "	>12½ "	40-50
	31	25	0	0	0	?	3 years	>6 mths.	>3½ "	35-65
	13	37	0	0	0	tuberculosis	1 year	>3 years	>4 "	30-40
	26	39	0	0	0	?	15 years	>5 "	>20 "	30-55

The 20 operated cases, as shown in Table 9, fall in 3 groups, namely:

Group I: 11 patients who have died.

" II: 2 patients whose condition was not improved by the operation.

" III: 7 patients showing very favorable result from the operation.

Some particular remarks about the three groups will be appropriate.

Group I comprises 11 cases who have died, 9 of them in close connection with the operation: 1 on the operating table, owing to a technical accident with perforation of the right ventricle (pericardial calcification was present); 1 died within a few hours after the operation; 5 died within 4 days after the operation; 1 died 6 weeks after the operation; and 1 died 2½ months after the operation. The remaining 2 patients in Group 1 survived the operation and the period of postoperative treatment but died respectively 4 and 6 years after. Their condition was not improved after the operation, which in one of these cases was not carried through completely. Thus the operative mortality is high. Presumably, however, this mortality may be lowered considerably through a more critical selection of the patients given operative treatment.

Group II comprises 2 cases, in which the result was uncertain. In one of them (Case 15) the condition has perhaps improved a little; in the other it has remained unchanged, but the post-operative observation period in these two cases is only 7 and 2 months respectively.

Group III comprises 7 patients in whom the operative result has to be designated as very favorable. In 3 of these cases (Nos. 5, 13 and 31) the symptoms disappeared completely. One of these patients (No. 13) was able after the operation to perform physical work without any difficulty whatever and even take part in a foot ball match; another patient (No. 31) was allowed to go through pregnancy and gave birth to a normal boy — without any labor complications whatever. The remaining 4 patients in this group improved quite considerably even though they did not become quite symptom-free, being unable to stand physical exertion quite as well as normal subjects. Prior to the operation, abdominal paracentesis had to be performed not infrequently in all these cases, and in no case has this been necessary after the operation; still, in 2 cases it was necessary after the operation to give theobromine for diuretic purpose. The period of survival after the operation is recorded in Table 9.

In order possibly to obtain more definite indications for operative treatment we have looked into the influence of various factors on the operative result.

1) *Age at the operation.* This is recorded in Table 9, and it ap-

pears not to be of particular importance as long as it is under 40 years. In Group I, perhaps, there is a preponderance of relatively old patients, but in all these a more probable explanation of the poor result than the age of the patient has been found, as 5 showed pericardial calcification, and 2 were suffering from tuberculous constriction of the heart; one of the latter was even in a very poor shape, and here the operation was resorted to as the ultimate refuge. With our present experiences we would hesitate in advising operative treatment for such patients. On the other hand, it may be said that two of the patients who died in close connection with the operation (Nos. 11 and 28) ought to have been able to go through this treatment, as here the indications for operative treatment were quite in keeping with those encountered in Group III, in which all the patients stood the operation very well.

2) *Pericardial calcification* was found in 7 of the operated cases. Of these 7 patients 5 died in connection with the operation, while 2 survived the operation without improving definitely (Nos. 24 and 15) and with a short postoperative observation period. Thus pericardial calcification is a very serious complication, implying a great operative risk and presumably only a slight chance of a favorable result even if the patient survives the operation — probably because in these cases it is difficult to make the pericardiolysis sufficiently extensive. Still, pericardial calcification is not to be looked upon as an absolute contraindication for operative treatment, as it may be difficult from the roentgenogram to decide the extent of the calcification and the resulting difficulties in pericardiolysis. Considering the poor prognosis without operative treatment it must be justified at any rate to perform an explorative thoracotomy and examine the practicability of pericardiolysis; but if the calcification implies some great difficulties, no further operative measures are to be taken.

3) *Arrhythmia perpetua* was found only twice among the operated cases, as beforehand it was looked upon as an unfavorable circumstance that has made us hesitate to recommend operative treatment in such cases (cf. Table 10). One of the operated patients with arrhythmia perpetua died 2 days after the operation; the other survived the operation, and later we succeeded in getting the rhythm regular, but otherwise the condition of this patient remained practically unchanged. Both of these patients presented also pericardial calcification.

4) *Enlargement of the heart* was found in 3 cases. One of these patients died 6 weeks after the operation (+ calcification), one showed no improvement after the operation (+ calcification), and one was absolutely much better after the operation (— calcification). So enlargement of the heart is no contraindication for the operative treatment.

5) *Etiology*. One patient with a traumatic etiology died (+ calcification). Of 3 tuberculous cases (no calcification) 2 terminated fatally, while one patient survived and became completely free from symptoms. Presumably a tuberculous etiology has to be taken as a warning, especially in the presence of pulmonary complications (see the last section, »Complicated Cases»).

6) As is evident from Table 9, presumably the preoperative duration of illness is of no importance. The decisive point is that the operation is performed before any severe degree of cardiac insufficiency has developed, and before the appearance of pericardial calcification, but obviously the rate at which this takes place is subject to great individual variation.

In setting up the indications and contraindications for the operative treatment we have to take into consideration also the chances of the patients without an operation. The fate of the non-operated patient is evident from Table 10, from which it will be noticed that 3 out of 5 patients in this group died respectively 3, 5 and 6 years after the diagnosis was made.

Table 10.
Various Data on Non-operated Patients.

Case No.	Age at diagnosis of illness	Pericardial calcification	Arrhythmia perpetua	Hypertrophy of the heart	Etiology	Duration of illness before diagnosis	Duration of illness after diagnosis	Total duration of illness	Died	Pulse pressure
7	28	+	0	+	Trauma	3 years	3 years	6 years	+	40—60
32	50	+	+	0	?	7 "	?	> 7 "	+	35—40
23	53	+	+	+	?	13 "	?	> 13 "	+	45—60
20	60	+	+	0	?	1 "	6 years	7 "	+	20—50
6	65	+	0	+	Coronary thromb.	4 "	5 "	9 "	+	60—90

The total lifetime after the onset of the symptoms for the 5 non-operated patients is 6—13 years, and it has to be taken into

consideration that this group represents the worst cases, namely: the patients to whom we did not dare to recommend any operative treatment. The contraindications for such treatment are plainly evident from Table 10: pericardial calcification, arrhythmia perpetua, and advanced age. From Table 9 it is further evident that several of the operated patients had had their symptoms for a number of years — even up to 15 years — and presumably without any operation these patients would also have had a good chance of living longer than the group of poor patients entered in Table 10.

So we have to reckon with the circumstance that patients with the syndrome of chronic constrictive pericarditis still have a chance of living for a number of years, which roughly may be estimated to lie between 1 and 10 years — in a few favorable cases, perhaps, even longer. There can be no doubt, however, that this lifetime is associated with complete disablement, and this fact suggests some guiding principles with regard to the indications for operative treatment. If the patients are relatively old persons, to whom it often is highly important to be able to keep living, albeit only for a few years (in order to be able to keep having their income, pension, etc. for the sake of their family and for education of their children), and to whom the capacity for physical work often is of minor importance, we have to be very reserved in exposing them to the great risk of death implied in pericardiectomy at this age. If, on the other hand, the patients are young persons to whom it is far more important to recover their working capacity, and for whom the operative risk is much smaller, this risk ought to be run, as such patients really have a considerable chance of a very favorable result. In the presence of pericardial calcification, however, the clinician should hesitate in recommending operative treatment to the young patients too, even though, we think, it will be advisable at any rate to perform an explorative thoracotomy.

Uncertain and Complicated Cases.

Finally we shall briefly mention the 8 uncertain and complicated cases, which are not included in the preceding account because they would considerably warp the presentation of the clear-cut cases.

In 4 of these cases the diagnosis was made at the autopsy; and it cannot be stated with certainty that the symphysis pericardii

revealed by the autopsy has given constriction of the heart, as the clinical examination was not sufficiently thorough with a view to this possibility, as there were also other anomalies sufficient to explain the cardiac insufficiency in these cases: mitral stenosis in 2 of the cases, thyrotoxicosis in 1, and enormous hypertrophy of the heart together with bundle branch block in 1 case. Before death, however, the last two cases had suggested the possibility of chronic constrictive pericarditis, in one case because the edemas would not subside under the treatment given; in the other case because no other reasonable etiological explanation of the heart lesion could be found.

In two other cases the diagnosis was merely ventilated but quite uncertain. One of these patients was also suffering from a mitral lesion.

In the last two cases the diagnosis of chronic constrictive pericarditis must be said to have been certain, even though in one case the constriction hardly was fully developed (only slight increase in the venous pressure). In both of these cases the lesion was of tuberculous etiology, but on thoracotomy it was found to be complicated with tuberculous empyema of the pleural cavity, on which account the performance of pericardiolysis was given up. Both patients died a few days after the operation.

From these cases, however, we still have learned that in cardiac insufficiency with pronounced edema in relatively young persons, in whom no diagnosis of the heart lesion seems obvious, we should always keep in mind the possibility of chronic constrictive pericarditis.

Summary.

A brief survey is given of the literature on chronic constrictive pericarditis, together with an account of a patient material comprising 25 clear-cut and typical cases of chronic constrictive pericarditis, besides 8 uncertain or complicated cases.

From the findings here presented it seems justified to conclude that patients suffering from this lesion ought to be given operative treatment if they are not too old. The presence of pericardial calcification urges strongly to great caution in the operative treatment and possibly limit the operation to an explorative measure. In cases of tuberculous etiology, the clinician will presumably have to be reserved in his employment of operative

treatment, demanding that the general condition of the patient must be good, and that there must be no evidence of active tuberculous processes.

In cardial insufficiency occurring in young persons in whom the etiological diagnosis is rather obscure, the clinician should always keep in mind the possibility of chronic constrictive pericarditis.

References.

- Beck, C. S.: The surgical treatment of pericardial scar. *J. A. M. A.* 97, 824, 1931. — Beck, C. S.: Remarks in discussion, see Harrington. — Boissin, R.: Contribution a l'étude de la symphyse cardiaque tuberculeuse. Thèse de Lyon, No. 1049, 1895. — Boutavant, L.: Des formes cliniques des symphyses cardiaques. Thèse de Lyon, No. 148, 1898 — 99. — Brauer, L.: Ueber chronische adhäsive Mediastino-Perikarditis und deren Behandlung. *Münch. Med. Wochenschr.* 49, 1072, 1932, 1902. — Burwell, C. S. & Blalock, A.: Chronic constrictive pericarditis. *J. A. M. A.* 110, 265, 1938. — Burwell, C. S. & Strayhorn, W. D.: Concretio cordis. *Arch. Surg.* 24, 106, 1932. — Bøggild, D.: Om fibros Mediastinopericarditis (fibros Pericarditis). *Ugeskr. f. L.* 99, 329, 1937. — Churchill, E. D.: Decortication of the heart (Delorme) for adhesive pericarditis. *Arch. Surg.* 19, 1457, 1929. — Churchill, E. D.: Pericardial resection in chronic constrictive pericarditis. *Ann. Surg.* 104, 516, 1936. — Cousin, J.: Contribution a l'étude anatomique et critique du foie cardiopulmonaire. Thèse de Paris, No. 47, 1899. — Delorme, E.: Sur un traitement chirurgical de la symphyse cardio-péricardique. *Bull. et mém. Soc. de chirurgiens de Paris* 24, 918, 1898. — Delorme, E.: Sur un traitement chirurgical de la symphyse du péricarde. *Gaz. d. hôp.* 1898, 1150. — Delorme, E.: Des conséquences de la symphyse cardio-péricardique; des indications d'une intervention directe. *Gaz. d. hôp.* 87, 341, 963, 1914. — Delorme, E.: Symphyse cardiaque et cardiolyse. *Progrès méd.* 39, 457, 1924. — Faber, K.: Pericarditisk Pseudolevercirrhose og dens Behandling. *Hospitalstidende* 12, 529, 1904. — Finsen, Niels R.: Om Behandling og Forebyggelse af Asciter. *Ugeskr. f. Læger*, 1894, p. 890, 909. — Finsen, Niels R.: Giver der en kronisk Klorernæringssygdom beroende paa en Ophobning af Saltet i Organismen? *Ugeskr. f. L.* 1904, pp. 145, 173. — Fløystrup, A. & Scheel, V.: Niels R. Finsens Krankheit. *Niels Finsens Mitteilungen über die Behandlung seiner Krankheit. Therap. d. Gegenwart* 46, 289, 1905. — Hallopeau, M. P.: Un cas de cardiolyse. *Bull. et mém. Soc. d. chirurgiens de Paris* 47, 1120, 1921. — Harrington, S. W.: Chronic constrictive pericarditis: Partial pericardiectomy and epicardiolysis in twenty-four cases. *Ann. Surg.* 120, 468, 1944. — Heuer, G. J. & Andrus, W. de W.: Diskussionsindlæg, see Harrington. — Heuer, G. J. & Stuart, H. J.: The surgical treatment of chronic constrictive pericarditis. *Surg., Gynec. & Obst.* 68, 979, 1939. — Holt, E.: Chronic adhesive pericarditis in childhood. *Am. J. M. Sc.* 178, 615, 1929. —

Hutinel, V. & Auscher dans Grancher, Comby & Marfan: *Traité des maladies de l'enfance*. Paris 1897 p. 223. — Ingvar, S.: Five cases of operated fibrous pericarditis. *Acta Med. Scand. Suppl.* 78, 278, 1936. — King, E. S. J.: *Surgery of the heart*. London 1941. — Lassen, H. C. A.: Om kronisk, fibros adhesiv Pericarditis (Symphysis pericardii). *Ugeskr. f. L.* 99, 567, 1937. — Lower, R.: *Tractatus de corde*. Amsterdam. 1669. 104—7. Cited after P. D. White. — Mohr, Th.: Et Tilfælde af Symphysis Pericardii med Levercirrhose hos et 13 Aars Barn. *Ugeskr. f. L.* 1902, pp. 385, 415. — Pick, F.: Über chronische unter dem Bilde der Lebercirrhose verlaufende Pericarditis (pericarditische Pseudo-Lebercirrhose) nebst Bemerkungen über die Zuckergussleber (Curschmann). *Zeitschr. f. klin. Med.* 29, 385, 1896. — Piquet, G.: De la symphyse de la péricarde. Conceptions actuelles cliniques, étiologiques et thérapeutiques. Thèse de Lyon 1939. — Rehn, L.: Die perikardialen Verwachsungen im Kindesalter. *Arch. f. Kinderh.* 68, 179, 1920. — Rehn, L.: Über pericardiale Verwachsungen. *Med. Klin.* 16, 991, 1920. — Santy, Bernheim, Piquet & Galy: La péricardite chronique constrictive. *La presse médicale* 47, 1676, 1939. — Sauerbruch, F.: *Die Chirurgie der Brustorgane*. Berlin. 1925. II. — Settergren, F.: Pericarditis fibrosa et calculosa och dess kirurgiska behandling. *Nordisk Medicin* 27, 1974, 1945. — Smith, E. S. & Liggett, H. S.: Cardiolytic for chronic mediastino-pericarditis. The 1928 Atlanta Proceedings of the inter-state postgraduate medical assembly of North America. — Smith, H. L. & Willis, F. A.: Pericarditis. *Arch. Int. Med.* 50, 171, 1932. — Sprague, H. B. & White, P. D.: The indications for and results of pericardial resection — the course of five cases. *M. Clin. Nth. Amer.* 15, 909, 1932. — Volhard, F. & Schmieden, V.: Über Erkennung und Umklammerung des Herzens durch schwierige Perikarditis. *Klin. Wochenschr.* 2, 5, 1923. — Warburg, E.: Traumatisk Hjertesygdom. Panserhjerte — Pick's Sygdom. *Nord. med. Tidsskrift* 6, 833, 1933. — Warburg, E.: Subacute and chronic pericardial and myocardial lesions due to non-penetrating traumatic injuries. Copenhagen 1938. — Warburg, E.: Om constrictio cordis. *Ugeskr. f. L.* 101, 433, 1939. — Westermann, H. H.: Die Ursachen der schwierig-schrumpfenden Herzbeutelentzündung und die Ergebnisse ihrer operativen Behandlung. *Arch. f. klin. Chir.* 205, 549, 1944. — White, P. D.: Chronic constrictive pericarditis (Pick's disease). *Lancet* 229, 539, 597, 1935. White, P. D. & Churchill, E. D.: The relief of obstruction to the circulation in a case of chronic constrictive pericarditis (Concretio cordis). *N. Eng. J. Med.* 202, 165, 1930.

From Ullevaal Hospital, Medical Department IX, Oslo.
(Chief: H. J. Ustvedt, M. D.)

Erythema Exudativum Multiforme.

II.

Relations to Tuberculosis.

By

HANS JACOB USTVEDT.

(Submitted for publication October 17, 1947.)

The author's interest in the correlation between Erythema multiforme and tuberculosis was aroused in 1935 through observation of a characteristic combination of phenomena: A 35-year-old woman with typical papulovesicular *E. multiforme* showed vesiculous reaction to tuberculin with appearance of lichenoid papules around the Pirquet scratch. *Hilar adenitis* was noted at the same time. A fortnight later she got a typical acute *polyarthrititis*. *Tubercle bacilli* were found in gastric lavage fluid and two months later she got exudative *pleuritis*. *E. multiforme* in this case appeared in exactly the same manner as *E. nodosum*, namely, as a signal to announce a tuberculous primary infection.

Considering the broad space the discussion of the genesis of erythema nodosum has occupied in the literature and the emotional vigour with which it has been conducted, it is remarkable to see how little attention has been devoted to the relation of erythema multiforme to tuberculosis. Most authors merely mention tuberculosis on a line with a long series of uncertain etiological possibilities. In Ormsby's »Diseases of the Skin», published in 1945, tuberculosis is not mentioned at all.

Ramel in 1929 reported a case of *E. multiforme* of tuberculous

ERYTHEMA EXUDATIVUM MULTIFORME.

genesis. Rotnes in his work on *E. nodosum* in 1936 shows that the 16 cases in which *E. nodosum* was combined with *E. multifforme* on the upper extremities, on the neck and face, bear an equally close relation to tuberculous primary infection as does the unmixed *E. nodosum*. Thune Andersen in 1937 described two cases of *E. m.* »with subsequent tuberculosis». In one case there was found a doubtful enlargement of the hilum, in the other a tuberculous affection of the apex was observed five months later. The cases by no means justify the conclusion that was drawn, namely, that »it seems as if *E. m.* is an early tuberculous manifestation, similarly to *E. nodosum*».

In the many reports of cases with severe affections of mucous membranes published in recent years the possibility of tuberculous etiology is as rule not mentioned. Jersild (1946) in his 25 cases finds no sign of tuberculosis. X-ray examination was carried out in eleven of these cases, gastric lavage in five.

N. Skiöld in his work on *E. nodosum* (1945) maintains that *E. multifforme* bears quite the same relation as *E. nodosum* to tuberculous primary infection and to other etiological possibilities. In both forms of exanthema he finds that barely two thirds of the cases have a tuberculous etiology and that 95 per cent of these are associated with the primary infection. As Skiöld, however, has adopted a diagnostic starting-point which in the present author's opinion is not tenable, seeing that he employs a much too restricted definition of *E. n.*, and has practically no genuine cases of *E. multifforme* in his material, his conclusion can hardly be accepted as valid.

In his work on the etiology of *E. nodosum* (1946) Löfgren reports a case of *E. multifforme* with fatal issue owing to pulmonary embolism. The patient showed *intra vitam* vesiculous tuberculin reaction, pulmonary changes indicative of fresh tuberculosis, growth of tubercle bacilli in the gastric lavage fluid and subsequent pleuritis. Autopsy revealed a caseous primary focus, caseated hilar lymphomas and a string of lymphomas from the hilum up to the upper cervical glands. The upper cervical lymphomas showed non-specific inflammation, while those lower down showed, in addition thereto, tuberculous changes of increasing extent towards the hilum. β -hemolytic and α -hemolytic streptococci were cultured from all the lymphomas and tubercle bacilli from the paratracheal and hilar lymphomas. This was probably a case of multiple infection by tubercle bacilli and streptococci, such as

Löfgren has clinically demonstrated in a number of cases of *E. nodosum*, on the basis of Westergren's investigations.

In his material of 178 cases of *E. n.* Löfgren has 6 cases with simultaneous occurrence of *E. m.* Three of these were found to be of tuberculous etiology, two were presumably due to streptococcal infection, in one cases were found false positive seroreactions for syphilis and in one case bilateral hilar adenitis with negative tuberculin reaction.

It is thus sufficiently clear that *E. multiforme* may arise in connection with tuberculous primary infection. The question is how often this may be the case and whether there is a possibility of distinguishing such cases on the basis of the clinical picture.

The Tuberculin Tests.

Of 202 patients in the present material 184, or 91 per cent, were tuberculin-tested. In two thirds of the cases only *one* Pirquet test was made, in one third two Pirquet tests or Pirquet + Mantoux 1 mg. This weakness in the technique can hardly be of much significance in this special connection. Löfgren is probably right in stating that, as regards the relation of such affections to tuberculous primary infection, the dividing line does not run between positive and negative reaction but between negative or faintly positive reaction on the one hand and strongly positive (vesiculous) reaction on the other hand. Individuals who react negatively to *one* Pirquet test have at any rate no high cutaneous sensitivity to tuberculin.

The fact that 72 per cent of the patients examined, including all age classes, reacted positively is in itself not very important. But it is of interest to compare the situation in *E. nodosum*, which is admitted to be in most cases associated with tuberculous primary infection, even if there may be differences of opinion as to how high the percentage of non-tuberculous cases of nodal fever may be. (Rotnes holds that the great majority of the cases are tuberculous, Skiöld mentions 60.5 per cent, Löfgren 58.4 per cent. Ustvedt: 60—70 per cent!) In the different investigations published respecting *E. nodosum* the percentage of tuberculin positivity is found to be surprisingly constant at 90—95 per cent, even in childhood, where the percentage of infection is otherwise now very low in most places (in our country 10—20

per cent). For *E. multiforme* we find, as is seen, positive reaction in only 72 per cent of the cases, although the material in the main consists of adults and even elderly individuals. This finding alone goes to indicate that, if there is any connection with the tuberculous primary infection, it is far from being such a dominant feature as in *E. nodosum*.

Table 1.

Tuberculin tests. Percentage of positive reactors.

0—9 years	3 of 9
10—19 "	10 " 21 (50 %)
20—29 "	31 " 52 (60 %)
30—39 "	43 " 56 (70 %)
40—49 "	20 " 24 (80 %)
50—59 "	11 " 12 (90 %)
60—69 "	2 " 7
70—79 "	2 " 3

Table 1 shows the percentage of reaction in the separate age groups. The figures are small, but they give us an indication of the tendency. In the age classes below 30 years not more than half of the patients react positively, and the percentage of reaction then rises with advancing age. The material is too heterogeneous (coming from town and country) and too small to admit of comparison with the average percentage of reaction in healthy subjects.

If we now regard *the separate groups* comprised in the material, a distinct and characteristic difference is revealed. In the group with mucous membrane affections (70 patients examined) 52 per cent react positively, in the group with simultaneous occurrence of *E. n.* efflorescences (54 examined) no less than 98 per cent show positive reaction. The group with pure *E. multiforme* without symptoms from mucous membranes occupies an intermediate position.

The *vesiculous* reactions show a similar distribution. 17 per cent of the positive reactions are vesiculous in the mucous membrane group, 30 per cent in the group with unmixed *E. m.* and 49 per cent in the group with combined *E. m.* and *E. n.* The difference is found to be the same if we examine separately the patients under 40 years old in order to eliminate the age difference in the groups.

The cases of pure *E. m.*, both with and without manifestations

from mucous membranes, show a characteristic difference between male and female patients, since 65 per cent of the females under 40 years old react positively, as against only 37 per cent of the males. The group with combined E. m. and E. n. consists, as already mentioned, almost exclusively of female patients.

From the results of the tuberculin tests it should then be permissible to assume that tuberculosis has some etiological significance in the group with combined E. m. and E. n. Further, the remarkable frequency of vesiculous reaction goes to indicate that it is tuberculous *primary infection* that here comes into play. Possibly we may also expect to find that the group with pure E. m. without mucous membrane manifestations has a somewhat higher degree of relationship to tuberculosis than the cases with such manifestations.

Tuberculous Primary Infection.

Definite signs of tuberculous primary infection consist in changes from negative to positive tuberculin reaction with detection at the same time of tubercle bacilli in expectorate or gastric lavage fluid. These criteria will be satisfied only under specially favourable conditions of observation, and such conditions are found only in some few cases in the present material.

Meanwhile it may be said that the combination of vesicular tuberculin reaction with radiographically observed changes of the primary tuberculosis type in lungs and hilum is strongly indicative of recent tuberculous primary infection. By the term «*probable* primary infection» I have here meant cases with vesiculous tuberculin reaction + typical hilar adenitis with polycyclic lateral demarcation on the radiogram, some times with perihilar condensation or a fresh pulmonary focus (primary complex). By the term «*possible* primary infection» I have designated cases with vesiculous tuberculin reaction, where the radiographic findings were judged by the radiologist to indicate hilar adenitis, but where the sharp lateral demarcation was lacking. It must be pointed out that vesicular tuberculin reaction alone, without characteristic radiographic findings, does not permit of any conclusions whatever with respect to primary infection.

In altogether 23 patients out of 202, or over 10 per cent, there were together with E. m. found signs of probable primary infec-

tion, and in a further 12 cases signs of possible primary infection, making in all 35 cases, or 17 per cent of the material. These figures lie considerably lower than those noted for *E. nodosum* (50—90 per cent), but are undoubtedly a good deal higher than what would be found in a normal material comprising the same age classes.

Likewise here there is seen a characteristic difference between the various groups. In the group with symptoms from mucous membranes there was not found a single indubitable case of tuberculous primary infection, and only three cases of possible primary infection, *i. e.*, 4 per cent. In the group with combined *E. m.* and *E. n.*, on the other hand, there were altogether 26 cases of probable and possible primary infection (18 + 8), that is to say, nearly half (47 per cent) of the 55 cases in the group. The group with pure *E. m.* without mucous membrane manifestations occupies, here as always, an intermediate position, since 5 out of 67 cases showed typical primary infection and 1 case possible primary infection, making together 9 per cent.

Especially remarkable is here the contrast between the cases with mucous membrane symptoms and the cases with some *E. nodosum*-efflorescences together with *E. multiforme*. It must here be taken into account that radiographic examination of the lungs was made in only one half of the cases in the former group, whereas in the latter group all the patients, except one, were radiographed. But the majority of the non-radiographed patients have been tuberculin-negative, so that this circumstance cannot be said to play any decisive rôle. On comparing with Skiöld's and Löfgren's figures it will be found that, alike as regards the percentage of tuberculin reactions, the number of vesiculous reactions and the number of probable and possible primary infections, the cases with combination of typical *E. multiforme* and *E. nodosum* are rather closely analogous to cases of *pure E. nodosum*.

I shall here once more point out that the group I have designated »combined *E. m.* and *E. n.*» does not comprise cases of dominant *E. nodos.* with some separate *E. multiforme* efflorescences, but that the reverse was the case: The clinical diagnosis was *E. multiforme* of the typical form with bullae, vesicles or cockade arrangement, with typical localisation, but at the same time there were found some separate red and tender nodules, sometimes very few in number, on the extension side of the legs, of the type seen in *E. nodosum*. I have previously mentioned that cases of *E.*

nodosum with admixture of some *E. multiforme* efflorescences seem to bear the same relation to tuberculous primary infection as the cases of pure *E. nodosum*. The present material seems to indicate that cases of dominant *E. multiforme* with admixture of some *E. nodosum* efflorescences stand in the same position. *Also the entirely unmixed E. multiforme, without a single E. nodosum nodule, may accompany the tuberculous primary infection.* In addition to the five cases noted in this material, in three of which tubercle bacilli were found, I have after conclusion of the investigation observed a further two typical cases of pure *E. multiforme* with positive tuberculin reaction, hilar adenitis and presence of tubercle bacilli.

It accords with the distribution of primary infections in the different groups that signs of earlier undergone tuberculous infection or disease were more frequently found in mucous membrane group (13 out of 80 cases) than in the group with combined *E. m.* and *E. n.* (6 out of 55). The figures are here very small.

Follow-up investigations of this material have not been made. But through re-admissions to the hospital it has been ascertained that 7 of the 23 patients with probable primary infection incurred other tuberculous diseases in the course of the first two years, including three cases of cavernous pulmonary tuberculosis. In two cases there was destruction in the primary infiltration itself, *a destructive primary tuberculosis*, such a Frostad has observed in a number of cases of *E. nodosum*.

I also wish to mention, as a matter of practical importance, that in two cases tubercle bacilli were found in the gastric lavage fluid together with entirely negative radiographic findings.

The question whether *E. multiforme* may also have relation to *later phases* in the course of a tuberculous infection has, here as in cases of *E. nodosum*, not been made clear. Wallgren's theory respecting the occurrence of post-primary *E. nodosum* after acute infections, with temporary tuberculin anergy, when the reaction changes over from negative to positive again, cannot be said to have gained support in the latter-day investigations. Löfgren advances good arguments for the view that in such cases the fact is rather that the *E. nodosum* has been called forth *by the acute infection*. In the present material *E. multiforme* occurred in 31 patients who showed signs of having previously undergone tuberculous infection (tuberculous disease, previous positive tuberculin reaction, calcified primary complex). Only in two of these

Burwell & Blalock take tuberculosis to be the most frequent cause of chronic constrictive pericarditis, stating that 16 of their 19 patients were suffering from tuberculous constrictive pericarditis. In most other materials the frequency of tuberculosis is recorded as being much lower — about 15 %. Some cases have been of traumatic origin, and in a few cases the syndrome has developed after coronary occlusion. Generally, however, the view prevails that in most cases the etiology of the lesion is quite obscure.

Patient Material.

For further elucidation of the syndrome we have considered it worth while to give a survey of the patients treated for this lesion in the Medical Department B of the Rigshospital, Copenhagen, since Warburg was appointed chief of this clinic.

Our present patient material comprises altogether 33 cases. Of this total, however, 8 have been somewhat uncertain or complicated, leaving thus 25 clear-cut cases of this lesion. The accuracy of the diagnosis has been established in 1 case by the highly typical clinical features of the syndrome. In the remaining cases the accuracy of the diagnosis was established not only by the typical clinical symptoms but also by the operative findings (19 cases), the roentgenographic findings (4 cases) and the autopsy findings (1 case).

The distribution of the cases on the various years of the period here concerned is shown in Table 1.

Table 1.

Distribution of the present Cases on the various Years.

1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	$\frac{1}{1} - \frac{1}{3}$ 47
2	0	1	2	2	0	0	1	3	1	3	0	2	6	2

From Table 1 it will be noticed that the first 15 cases are distributed fairly equally over the years of 1933—1944, while in the first 2 years after the cessation of the world war no less than 10 patients were admitted.

This material is made up of 18 men and 7 women.

The age distribution of the material is given in Table 2.

Six patients with severe exanthema and mucous membrane affection showed negative tuberculin reaction during the exanthema stage, but shortly afterwards typical positive reaction, in other words: a *transient anergy*, such as is seen in many acute illnesses. This finding speaks distinctly against tuberculous etiology in these cases.

One patient had, when 20 years old, had *E. nodosum* with exudative pleurisy, a combination which in the great majority of cases seems to be due to tuberculous infection. 27 years later she got *E. multiforme*. Pirquet was vesicular, and radiographic examination revealed a typical hilar adenitis. This may have been of non-tuberculous origin, but we cannot exclude the theoretical possibility of a genuine *reinfection* in a case where the original tuberculous infection had become extinct. Another patient with typical calcified primary complex showed during an outbreak of *E. multiforme* vesicular tuberculin reaction with certain hilar adenitis.

It is well known that in rare instances both the exanthema in *E. nodosum* and the hilar adenitis may be observed before sensitivity to tuberculin has been noted. In a woman aged 26 there was found together with *E. multiforme* a hilar adenitis with exudative pleurisy. Pirquet was three times found to be negative and did not become positive until after 18 days. Tubercle bacilli were found in gastric lavage fluid. More uncertain was the situation in this respect in a 25-year-old woman who in March had exudative pleurisy with presence of tubercle bacilli and in June the same year got pure *E. multiforme* without symptoms from mucous membranes.

In all 36 cases of certain or probable hilar adenitis with vesicular tuberculin reaction the adenitis was *unilateral*. In 1939 the author pointed out that the unilateral hilar adenitis is, in its radiographic features, highly characteristic of tuberculous primary infection, whereas *bilateral* hilar adenitis is only seldom to be ascribed to tuberculosis, but is occasionally due to Boeck's sarcoid, or to unknown conditions. In the same year J. H. Vogt described a combination of joint-pains, erythema nodosum and bilateral hilar adenitis in a tuberculin-negative patient. In the present material there is found a case of typical *E. multiforme* in a 44-year-old woman with negative Pirquet and Mantoux, severe joint-pains and bilateral hilar adenitis. Löfgren has a similar case in his material. There is no particular reason for assuming that this

cases was the exanthema immediately preceded by an acute infectious disease, in both cases an angina.

In a couple of cases E. m. appeared as *a link in a chain of tuberculous manifestations*. Example: A 20-year-old woman had first a tuberculous anal abscess, 8 months later erythema nodosum, 6 months after this a recurrence of the anal abscess, at the same time E. multiforme with some few E. nodosum nodules, pulmonary infiltration and vesiculous tuberculin reaction. Three years later fully developed cavernous phthisis.

There is reason to devote particular attention to *the result of the tuberculin test* in cases of E. multiforme. Occasionally there may be seen already in the first 24 hours typical E. multiforme efflorescences around the Pirquet scratch, with papules and vesicles. It is important to bear in mind that this does not necessarily imply a positive, specific reaction, since this phenomenon may also appear in cases where subsequent careful control tests with tuberculin yield negative results. The same typical efflorescences may be seen around a control scratch made without use of tuberculin. Sometimes a large bulla is found around the Pirquet scratch, and I have also seen bleeding therein in case of a tuberculin-negative patient with hemorrhagic E. m. Sure conclusions can in such cases be drawn from the tuberculin test only after the eruption has disappeared.

On rare occasions we find in patients with E. m. a generally increased cutaneous sensitivity, which finds expression in positive reaction to a number of specific and unspecific products, as Owren, for example, has shown in a case from Medical Dept. A of the Rikshospital. Such cases seem to represent exceptions. Rotnes in several of his cases made investigations as to the reaction to NaCl, milk, broth, serum, staphylococcal and streptococcal emulsions, trichophytin, and found no general increase in cutaneous sensitivity. Likewise Löfgren's results indicate that in the great majority of cases the reactions to tuberculin or to streptococcal emulsion are specific, and I have come to the same result in a large number of cases. Great cutaneous sensitivity is stated to be characteristic of *Behcet's syndrome*. According to T. Jensen, however, the sensitivity here differs somewhat from that mentioned above, seeing that there comes a pustule 24 hours after puncture with a needle in skin or mucous membrane, while no reaction is obtained by scarification of the epidermis. H. Rygh found no pronounced cutaneous sensitivity in his case of Behcet's syndrome.

to recurrence. I shall revert to this matter when discussing the etiology in its entirety.

Summary.

Tuberculin tests are performed in 184 of 202 patients with erythema multiforme. 72 % showed positive reaction. In the cases with affections of the mucous membranes 52 % showed positive reaction, against 62 % in the cases with pure *E. multiforme*, and 98 % in the cases with combined *E. multiforme* and *E. nodosum*.

Signs of probable tuberculous primary infection were found in 23 cases of 202, signs of possible primary infection in 12 cases. In the group with affections of the mucous membranes no case of probable and only 3 cases of possible primary infection were found, *i. e.* 4 %. In pure *E. multiforme* 9 %, and in the combined group 47 % (26 of 55 cases) showed signs of probable or possible primary infection. Certain details concerning the relation between tuberculosis and *E. multiforme* and the behaviour of the tuberculin reaction in *E. multiforme* are discussed.

Tuberculosis seems to be an important etiological factor in *E. multiforme* combined with *E. nodosum*, and plays a certain rôle in pure *E. multiforme* without symptoms from the mucous membranes, but seems to be without importance in cases with affections of the mucous membranes, except in cases with episcleritis.

From the Medical Clinic of the University of Lund, Sweden.

On the Artificial Kidney III

Technical and Methodological Problems.

By

NILS ALWALL and LEMBIT NORVIIT

with the collaboration of

A. M. STEINS.

(Submitted for publication October 20, 1947.)

Earlier papers of this series gave an account of (a) the principle and the constructional details of the apparatus for the dialysis of the blood (A. 1947) and (b) the results concerning the effectivity of the apparatus in dialyzing the blood in vitro and in vivo (A. and N. 1947).

In the following some technical and methodological details of general interest for dialysis treatment are reported.

The Apparatus.

The apparatus consists of two cylinders of wire netting around which is wound cellophane casing enclosed by mantles of wire netting. The cylinders are totally submerged in a glass container filled with a salt solution. A lid prevents evaporation and at the same time permits the saturation of the solution with a suitable gaseous mixture. A motor driven propeller stirs the solution continuously.

The Cellophane Casing: After trying various makes we found the cellophane casing supplied by The Visking Corporation, Chicago (Visking Casing 27/32") to be the most suitable. This casing is of uniform width, it is flexible and practically non-stretchable; it is impermeable to plasma proteins.

This casing is generally delivered in large rolls. This sort of packing, however, seems to favour breakage of the casing owing to a slipping

of the convolutions. Time and material can thus be wasted before it is possible to find a flawless piece 5—6 metres long, *i. e.* the length necessary for each cylinder. The Visking Corporation has now, at our request, supplied the cellophane casing in smaller, flanged reels. It seems that the above inconvenience is thereby now practically eliminated.

The mounting of the Cellophane casing: Blood enters and leaves the casing via glass tubes. A piece of thick, soft rubber tubing is threaded on the glass tubes at the points of connection. The moistened casing is fastened to the glass tube and the intermediate rubber tubing by means of several moistened silk or cotton threads that are most carefully wound tightly round the tubing.

Test of the tightness of the casing: When the cylinder has been mounted with the casing and the network mantle, the casing is tested for air-tightness by inflation to artery pressure. During this test the cylinder is kept submerged in water.

After sterilization in an autoclave the warm cylinder must be submerged in a liquid immediately, because the casing will otherwise dry up quickly and can break.

If the apparatus is not used immediately after sterilization, we generally replace the casing with a new one.

Before using the apparatus, the casing is again tested in the following manner. The casing is rinsed with a sterile salt solution to expel the air. It is then filled to arterial pressure with a salt solution. If the pressure remains constant, *i. e.*, the liquid does not escape from the tubing at this pressure, the apparatus is ready for use (Fig. 1, IV).

Protein will not escape from a flawless casing. The salt solution will thus give *e. g.*, a negative sulphasalicylic acid reaction, when dialyzing blood *in vivo*. If there is any protein in the salt solution, the motor driven propeller will cause it to froth.

Expulsion of air from the cellophane casing: There is of course an air-clot-catcher coupled between the apparatus and the patient, but it is desirable that the casing be as void of air as possible *inter alia* because air decreases the dialytic area. The following technique, illustrated in Fig. 1, is used to expel the air from the casing. The casing of the assembled apparatus is connected to a bottle with an air pump. From the bottle sterile salt solution is pumped through the cellophane casing (Fig. 1, I). This drives out the greater part of the air from the casing. The salt solution

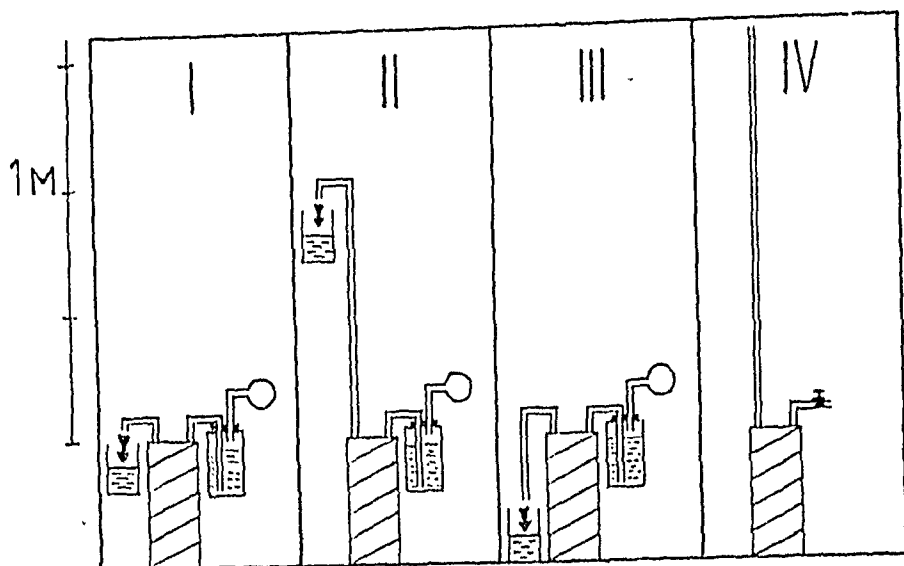


Figure 1. A. Expulsion of air from the sterile cellophane casing: I. From the bottle sterile salt solution is pumped through the cellophane casing. II. The salt solution flows from the apparatus through a glass tube about 1 meter high. III. The salt solution flows off through a lower glass tube.

B. Test of the tightness of the sterile casing: IV. The casing is filled to arterial pressure with a sterile salt solution. If the pressure remains constant, i. e., the liquid does not escape from the tubing at this pressure, the casing is tight.

is then allowed to flow from the apparatus through a glass tube about 1 meter high (Fig. 1, II). After a while, when the pressure drops again owing to the fact that the salt solution is allowed to flow off through a lower glass tube, the sudden decrease in pressure will force out the air (Fig. 1, III). Such changes in pressure are repeated a few times.

Afterwards the current of the fluid is reversed by coupling the bottle with the pump to the other connection tube of the cellophane casing. The above mentioned process with changes of pressure is repeated a few times. In this manner practically all the air is expelled. A small quantity of air may remain in the casing without following the flow of blood during treatment.

The network mantle is in principle a new and important constructional detail. The casing is compressed between the network of the mantle and the cylinder so that the blood is continually spread in a thin layer along the whole of the casing, whose content is thus small and independent of the pressure exercised by the blood.

As it might be difficult for inexperienced hands to fit the mantle onto the cylinder without damaging the casing, we have tried to find a softer

material than wire netting, but owing perhaps to the present difficulties in getting materials we have so far been unsuccessful. Ordinary gauze for instance, decreases the rate of dialysis — as expected — and can easily cause a local compression of the casing with a consequential disturbance of the flow of the blood.

Stirring of the salt solution. The salt solution is stirred by means of a motor driven propeller. The number of r. p. m. for a maximal dialysis must of course be determined separately for each apparatus, *e. g.*, in tests with urea (the authors, 1947). A gramophone motor, which is both cheap and silent, is powerful enough to stir a salt solution in our apparatus with a capacity of about 25 litres salt solution and intended for homo.

Renewal of salt solution: A thick pipe extends almost to the bottom of the glass jar of the apparatus. To this pipe is connected a thick rubber tube whose free end is deeply submerged in an outlet tube. The glass jar can be emptied in a couple of minutes. In an adjacent laboratory there is a container for the fresh salt solution that is maintained at a suitable temperature. From this container the solution is conducted through a pipe in the wall to the apparatus.

When treating uremia we generally change the salt solution roughly every two hours. By this time the non-protein nitrogen in the solution has as a rule risen to at most $1/10$ — $1/5$ of its concentration in the blood flowing to the apparatus. We thus maintain a great difference between the concentration inside and outside the casing, which favours the rate of dialysis.

Dialytic area: In our apparatus the total dialytic area of the two cylinders is about 6,500 cm² with a casing length of 10—11 metres. This size is suitable for a flow of about 8—10 litres of blood, which is probably the greatest quantity that can be drawn from the arteria radialis under these conditions. As mentioned in an earlier publication by the present authors (1947) an apparatus of this capacity gives a satisfactory dialytic effect: *it is sufficient substantially to remove any uremia within 24 hours' treatment.*

Of our experimental results obtained with smaller dialytic areas, those will now be mentioned where the surface was reduced to about 55—60 % of that of the above, *i. e.* approx. 6—6.5 metres of casing (Fig. 2—3). The yield is expressed as a percentage of that obtained in earlier tests with a 10—11 metres casing and with corresponding rates of flow of 500 mg % urea solution (the present authors 1947).

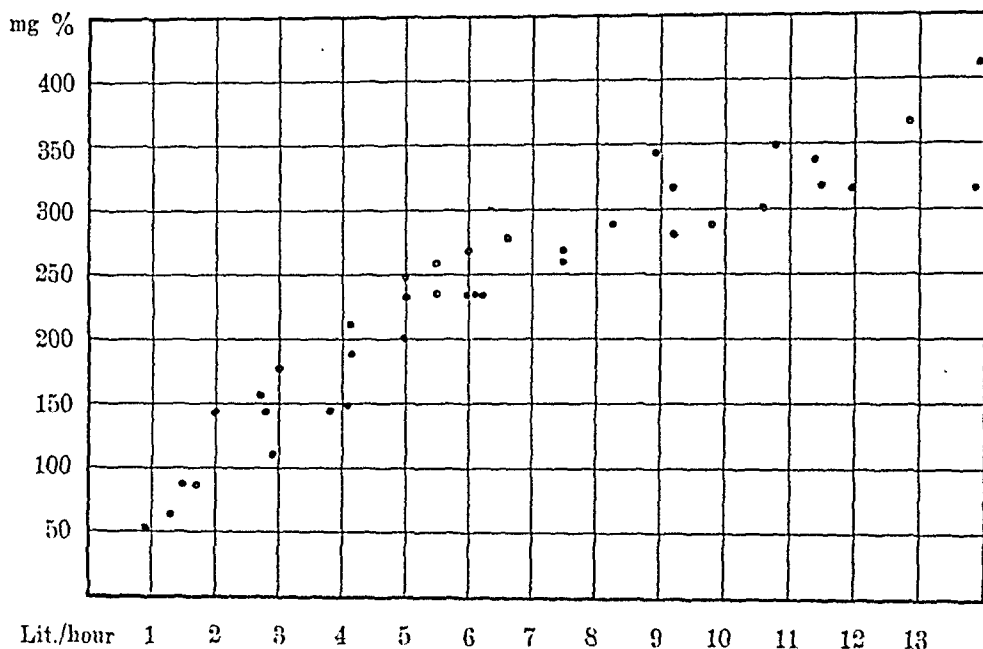


Figure 2. Apparatus for homo, 6—6.5 m cellophane casing. The urea concentration in the liquid which was conveyed to the casing was 500 mg%. The figure shows the separate observations of the urea concentration in the liquid which leaves the casing. The x-axis gives the flow speed of the urea solution passing through the casing in litres per hour and the y-axis refers to the urea concentration in the solution which leaves the casing.

Thus we have found that with lower rates of flow the dialytic effect is little lower when using the shorter casing. With higher rates of flow, with those practicable for the dialytic treatment of homo, the dialytic effect does not fall in proportion to the decrease in the area of the dialytic surface. Thus it may be possible to obtain about the same dialytic effect by using only the big cylinder (6—6.5 metres of casing) and by corresponding prolongation of the time of dialysis.

The Salt Solution.

When carrying out dialytic treatment Kolff, 1946, first used Darrow's »interstitial solution»: 0.65 % NaCl, 0.25 % NaHCO₃ and 0.018 % KCl. Later Kolff used a solution of the following composition: 0.6 % NaCl, 0.2 % NaHCO₃, 0.04 % KCl and 1.5—2 % glucose in tapwater, whose calcium content was 4 mg%. Various modifications of common serum salt solutions have been employed also in dialytic experiments by earlier authors.

In Kolff's apparatus, the big cylinder, around which the casing is wound, rotates above the surface of the solution, except for the lower part of the cylinder, which is submerged in the salt solution with which the container is filled. It is therefore impossible to regulate the H-ion

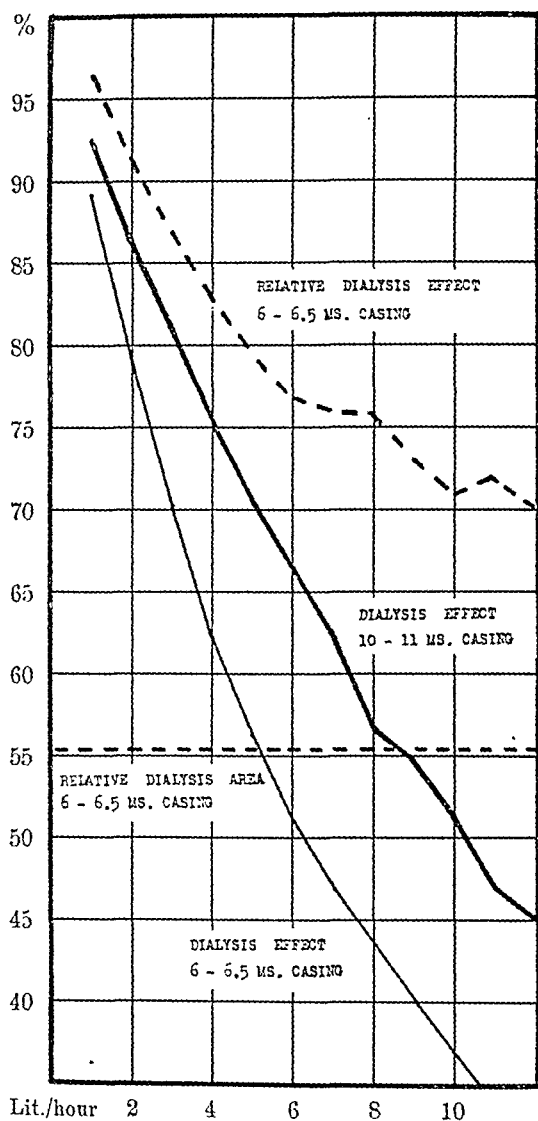


Figure 3. Apparatus for homo. 500 mg% urea solution. Some 11 or 6—6.5 m cellophane casing respectively. The figure shows (a) The percentage amount of the supplied urea (y-axis) which is dialysed away at different flow speeds (x-axis) with a casing length of about 11 metres (quoted from Alwall and Norviit, 1947) and 6—6.5 m respectively. (b) The yield obtained with 6—6.5 m of casing expressed as a percentage of that obtained with about 11 metres casing and with corresponding rates of flow.

concentration of the solution by means of sodium bicarbonate + CO_2 . There is a considerable evaporation from the surface of the solution, whose temperature is about 40°C . The loss of water incurred by this evaporation must be replaced now and then. This means that the concentration of the solution cannot be adjusted with desirable accuracy. Moreover, a certain amount of the solution is splashed out by the rotation of the cylinder. As the conditions under which dialysis is performed do not permit calcium to be maintained in a dissolved state in the salt solution, Kolff gives his patients calcium intravenously during dialytic treatment.

The present apparatus is furnished with a lid, which practically prevents evaporation — less than 1 per cent loss of water for a five hours' dialysis without a change of the saline solution — and permits pH regulation by means of CO_2 —bicarbonate.

We have found the following composition suitable for the salt solution: 0.65 % NaCl , 0.025 % CaCl_2 , 0.025 % KCl , 0.015 % NaH_2PO_4 , 0.01 % MgCl_2 , 0.23 % NaHCO_3 and 0.1 % glucose in distilled water. During dialysis 6.5 % CO_2 in O_2 is bubbled through the salt solution. The temperature is maintained at about 38°C .

First the other salts are dissolved and then the bicarbonate. When the salts have been dissolved and the solution saturated with the above mentioned gaseous mixture, CaCl_2 is added. In this manner there are no difficulties in keeping the calcium dissolved.

Also in cases of prolonged dialysis of blood against this solution, which were carried out in vivo in a large number of experiments on animals and in vitro, no alteration of practical importance in the composition of normal serum could be observed.

Fluid Balance Blood—Salt Solution.

If blood is present inside the casing and salt solution outside, the blood will be diluted by osmotic water attraction.

Kolff tries to counteract the colloidal osmotic pressure of the blood by giving the salt solution a high glucose concentration.

In earlier experiments on the dialysis of the blood in vivo, it seems as if no attention was paid to the fluid-balance, possibly because the capacity of the dialyzers of those days was so small that this problem did not arise.

was a case of Boeck's sarcoid. I have not found any record of the occurrence of E. multiforme in connection with that disease. Otherwise I may refer to another publication on this subject (Ustvedt: Further Observations concerning Bilateral Hilar Adenitis).

Thus we find that E. multiforme in the cases where tender nodules on the legs are to be found at the same time can probably be placed on a line with E. nodosum with respect to the importance of tuberculous primary infection as etiological factor. Further it is clear that also the unmixed E. multiforme without such E. nodosum efflorescences is sometimes, although far more seldom, due to tuberculous primary infection. On the other hand, it is doubtful whether tuberculosis plays any part whatever as causal factor in cases with manifestations from mucous membranes. An exception is here represented by the cases with *episcleritis*, which seem to stand in the same position as cases of combined E. m. and E. n. Where the exanthema is of bullous type the possibility of tuberculous etiology is evidently very small.

In this connection I may call to mind the difference which in the introductory description of the clinical picture was shown to exist between »the mucous membrane group» and »the combination group» (E. m. + E. n.). In the former group there was a certain preponderance of male patients, while in the latter 95 per cent were females, as in pure E. nodosum. The tendency to recurrence is mainly to be seen in the first group. On the other hand, the cases with combined E. m. and E. n. far more frequently presented prodromes in the form of dysphagia and joint-pains, such as are also seen in E. nodosum. Likewise the high values found for the sedimentation rate were to a certain degree characteristic of this group.

As regards the pure E. multiforme without mucous membrane symptoms and without E. nodosum efflorescences, which seems in every respect to occupy an intermediate position between the other two groups, it is not unreasonable to suppose that we have here to do partly with cases that are of the same kind as the combination cases; which is evidenced by, *inter alia*, their association with tuberculous primary infection, partly with cases of the same nature as the mucous membrane cases, as is indicated by, for instance, the appearance of bullous exanthema and tendency

The fluid balance between the blood in the cellophane casing and the solution outside it may be obtained in two different ways: a) by adding quantities of a suitable colloid to the salt solution until its colloidal osmotic pressure is equal to that of the blood, b) by compensating the colloidal osmotic pressure of the blood by corresponding hydrostatic pressure.

1. Colloids.

When a colloid is added to the salt solution for dialyzing blood in vivo, the colloid is not administered intravenously, it is true, but the contact is indirect via the cellophane membrane. However, large quantities of blood come into such indirect contact with large quantities of salt solution (when treating homo, about 100—200 litres of blood to 200—250 litres of salt solution per uninterrupted treatment). Such colloids as are available in the open market and can be regarded as applicable for this purpose are, however, not uniform but contain molecules of different sizes. They ought therefore not to be used for the dialytic treatment of human beings before they have been most carefully tested on animals and, if necessary, cleansed of low-molecular compounds that might pass through the membrane, exercise a toxic effect on, or remain deposited in the organism of the individual undergoing treatment.

Besides the above disadvantages there are others that will be dealt with in the following.

In this respect our studies are confined to the dialysis of blood against colloidal salt solutions in vitro and to a limited extent to experiments on rabbits. We have tried *gummi arabicum* (acacia) and *amylum solubile*.¹ As was only to be expected, we had no difficulties in maintaining the fluid balance between the blood and the salt solution + colloid. The following experiences are however worthy of mention: a) These substances contain cristalloids of varying or non-physiological concentration; our preparation of *gummi arabicum* contained large quantities of calcium and *amylum solubile*, large quantities of phosphorus, b) The strong formation of froth made it difficult to control the pH value of the salt solution by means of sodium bicarbonate + CO_2 .

¹ By the courtesy of the Research Laboratory L. K. B. (polyvinyl-alcohol) and A/B Ferrosan (*amylum solubile*) who placed preparations at our disposal free of charge, it was possible for us to carry out this part of our research work.

Table 1.

The influence of the hydrostatic pressure on the liquid balance between heparinized animal plasma in the cellophane casing and the surrounding salt solution during 6 hours' dialysis.

Hydrostatic pressure cm water	Plasma protein concentration in per cent	
	before dialysis	change after dialysis.
5	6.86	— 0.70
5	6.40	— 0.40
10	7.55	— 0.45
15	7.55	— 0.35
20	7.72	— 0.50
25	6.60	— 0.25
30	8.06	— 0.55
30	6.40	± 0
35	7.30	± 0
39	6.86	± 0
45	7.20	+ 0.10
47	6.70	± 0
48	6.75	+ 0.20
55	7.35	+ 0.35

In vivo conditions are more complicated. The pressure exercised by the blood on the cellophane casing during its passage through the apparatus and other factors change in an elusive manner. On the whole however, the experiences illustrated in Table 1 may be utilized.

When experimenting on rabbits we place the apparatus on a lower level than that of the animal, generally so that the surface of the solution lies about 15 cm below the height of the heart. The blood is allowed to flow freely from an artery to the apparatus and back again into a vein. The weight of the animal is registered during the treatment. If necessary the difference between the level of the rabbit and that of the apparatus may be adjusted.

When dialyzing homo we have determined possible differences in liquid balance by measuring the changes in volume of the salt solution in the apparatus. We have so far not had any possibility of checking the weight of a bed patient during treatment. The construction of the bed is such as to allow necessary alterations of the level of the patient in order to adjust the liquid balance in a suitable manner.

On account of its construction Kolff's apparatus does not permit the utilization of hydrostatic pressure for controlling the fluid balance, a problem which, by the way, has not been mentioned by Kolff.

Hemolysis.

Dialytic treatment entails the passage of relatively large quantities of blood through the apparatus. The total quantity of a patient's blood can be passed through the apparatus about twice per hour. It is therefore of importance that the apparatus be constructed in a manner that will eliminate hemolyzing factors.

Kolff has a hemolysis problem that he tried to solve by adding 1.5 % glucose to the salt solution. However, K. later found that one considerable hemolyzing factor was too rapid a rotation of the cylinder. This rate was therefore reduced to 25 r. p. m. in spite of the fact that a greater speed seems to be necessary for a max. dialytic effect. Furthermore Kolff asserts that hemolysis can occur if the blood remains too long in the apparatus.

It would seem as if many mechanical factors in Kolff's apparatus might cause hemolysis: The blood has to pass two rotating couplings, the rotating cylinder, and the Beck tube pump. The length of the tube is no less than 40—45 metres.

When we began our experiments on animals we occasionally observed hemolysis when dialyzing. In view of this fact we made a systematic study of possible hemolyzing factors that might be present under the conditions of our animals experiments.

A Beck tube pump was first used as a volumeter and flow control. The pump was coupled between the artery and apparatus. It would seem as if the pump may, especially if the rubber tubing is of poor, war-time quality, cause hemolysis.

The pump has also another drawback: If the rate of inflow of the blood should for some reason or other be insufficient, the pump can damage the blood vessel.

A dropper connected to the circulation was for some time used as a volumeter. This can lead to hemolysis, especially if the fall of the drops is too great.

Vigorous stirring of the salt solution, several times greater than is necessary for max. effect of dialysis, will not cause hemolysis unless the apparatus is without a network mantle and the cellophane casing furthermore contains much air.

Variation of temperature is of no importance. Blood can be cooled on its way from the experimental animal to the dialysis apparatus where it can be heated up to at least 43° C and finally cooled down on its way back to the animal without incurring hemolysis.

The composition of the salt solution may be modified within physiological limits without the occurrence of hemolysis during experimental dialysis. Thus, no increase in the glucose content of the salt solution is

necessary for the prevention of hemolysis. Experience showed that it was also possible to add the above mentioned colloids to the solution without the appearance of hemolysis.

Nowadays we connect the apparatus direct to the artery. The arterial pressure forces the blood as quickly as possible through the relatively short casing and back to the vein. In this smooth-walled, closed system it is not necessary for the blood to pass any rotating couplings, rotating cylinder or rotating Beck pump.

Thus we have no hemolysis problem.

Determination of the Rate of Flow of the Blood.

We have described above some of our experiences of the following methods for determination and regulation of the rate of flow of the blood through the apparatus: 1) Beck pump, 2) Dropper and 3) a volumeter-valve of the kind described by one of us (A. 1947). By and by we learned that when carrying out experiments on animals, it was possible without risk to let the blood flow freely from the arteria carotis, through the apparatus and back to the vena jugularis. This method was afterwards applied on human beings where the blood is drawn from the arteria radialis. In this manner we obtain the advantage of a simplified dialysis technique.

For many reasons however, a certain knowledge of the rate of flow of the blood through the apparatus is desirable.

Fischer and Porter and Co., manufacture for industrial purposes a volumeter whose capacity might be suitable for the continual determination of the flow of blood through the apparatus during dialysis. Owing to financial reasons, however, we have not yet been able to try this volumeter. A venturimeter reduces the rate of flow and is not simple to handle.

We have been using the following methods for determination of the rate of flow of the blood through the apparatus:

(1) The blood leaving the apparatus is made to flow for some minutes — against a pressure corresponding to the venous resistance — into a graduated glass.

(2) A clamp or clip is fixed to the rubber tube through which the blood flows from the artery and the circulation is interrupted. Distally to the clip a suitable quantity of salt or glucose solution is injected through the rubber tube, which consequently forces

the blood from the cellophane casing to the vein and gives a colourless stretch. When the connection to the artery is again opened, the blood forces the colourless solution through the apparatus without any substantial intermixture (blood + solution) taking place. The time is taken from the moment the clip on the rubber tube by the artery is opened until the moment when the solution has passed and pure blood again begins to flow from the apparatus to the vein. With the knowledge of the volume of the cellophane casing, it is thus possible to estimate the rate of flow of blood through the apparatus.

(3) The whole stream of blood from the artery is conducted via a side-tube into an empty glass bottle. Thereby the air content of the bottle is expelled and passed into another glass bottle filled with blood. The air forces this blood on to the apparatus. As soon as the first bottle is almost full of blood and consequently the second bottle almost emptied, the connection between the bottles is cut off and the blood again allowed to flow from the artery direct to the apparatus.

As the bottles are graduated it is possible to read the rate of flow to or from them.

For the next determination, the order of the bottles can be reversed so that a new filling of the bottle with blood prior to determination will not be necessary.

(4) When experimenting on animals we generally obtain a rough conception of the rate of the flow of blood in the following manner: A mercury manometer is connected to the tube connecting the artery and the apparatus. When the connection to the apparatus is shut off distally to the manometer, the arterial pressure is registered. When the connection to the apparatus is opened again, the pressure drops. When the rate of flow from the artery is sufficient there will be a considerable difference between the pressures thus registered. An increase in the resistance in the apparatus and decrease in the rate of flow reduces the above mentioned difference.

Summary.

A report is given of our present solution of certain technical and methodological problems, which seem to be of fundamental importance and of practical interest for dialysis treatment.

Some points are explained by means of brief accounts of the experimental studies, upon which the present solution of the problem is based, or brief mention of the difficulties encountered in this sort of work.

The report refers to: 1) Manipulation of the apparatus, 2) Composition of the salt solution against which the blood is dialyzed, 3) Maintaining of the fluid-balance blood-salt solution, 4) The problem of hemolysis, and finally 5) A few remarks on the technique for determining the rate of flow of the blood through the apparatus.

Literature.

Alwall, N.: *Acta med. scand.* 1947, *128*, 317. — Alwall, N. and Norviit, L.: *Ibid.* 1947, *Suppl. 196*, 250. — Kolff, W. J.: *The artificial kidney*, Kampen (Holland) 1946.

From the Thyroid Clinic of the Massachusetts General Hospital
(Chief: Prof. J. H. Means) and the Radioactivity Center of the
Department of Physics, Massachusetts Institute of Technology
(Chief: Prof. R. D. Evans), Boston, Massachusetts (U.S.A.)

Radioactive Iodine: Its use in Studying the Urinary Excretion of Iodine by Humans in Various States of Thyroid Function.

A Preliminary Report.

By

BENGT SKANSE.¹

(Submitted for publication Sept. 5, 1947.)

In recent years the physicists have provided the physicians and biologists with a variety of new tools to be used in studying certain biological phenomena. One of the most important of these tools is the radioactive isotope technique.

It was in the year of 1923 that Hevesy first employed the natural radioactive isotope of lead, Radium D, as a tracer substance to study the metabolism of lead in plant physiology. While Joliot and Curie (1934) introduced a method to produce artificial radioactive isotopes, Hevesy pioneered with tracer technique in biology and medicine.

The fundamental basis for using radioactive elements as tracer substances is that these radioactive isotopes behave or at least should behave exactly in the same way as the inactive isotopes of the same element. As the chemical properties of an element are dependent upon the arrangement of the electrons around the atomic nucleus, and this arrangement is the same in radioactive as in inactive isotopes of the same element, we have very good

¹ Rockefeller Fellow 1946—1947. Henry P. Walcott Fellow in Medicine, Harvard University 1947—1948.

120 hour period to excrete in the urine between 73.9 and 88.5 percent of the administered radioactive iodine. Between 70.3 and 87.2 percent of the administered dose was excreted during the first 48 hour period. Two cases having myxedema were observed to excrete 91.4 and 89.1 percent of the radioactive iodine in a period of 120 hours and 76.0 and 76.8 percent in a 48 hour period, thus indicating a slower than normal renal excretion of the iodine. They observed that three patients having non toxic goiters and five patients having toxic goiters excreted about the same amount of iodine as that excreted by the normal subjects. The observation that the five patients having toxic goiters excreted the same amount of iodine as did the normal subjects is somewhat surprising. However this might be explained on the basis of previous treatment with iodine (Lugol's solution), which was stopped only 12 to 24 hours before administration of the tracer iodine to four of the five patients. It is also quite possible that the large carrier dose, 14 milligrams of iodine, acted to decrease the uptake of labelled iodine. This explanation is supported by the observation that the one patient who had had no previous therapy with iodine when given 14 milligrams of iodine labelled with radioactive iodine excreted 82 percent of the tracer in a 48 hour period. The radioactive iodine excretion in feces was very small, ranging from 0.06 to 2.82 percent, except in one case where the fecal excretion was 11.25 percent. This last finding may be due to a mixing of feces and urine. In one patient they measured the radioactivity directly over the thyroid gland by means of a gamma ray Geiger-Müller counter.

In a subsequent report Hamilton and Soley (1940) used this in vivo measurement technique to study patients with various types of goiters. In this study also they administered as large a dose of iodine as 14 milligrams. The radioactive iodine used to label this inert iodine was equivalent to 12 to 50 micrograms of radium element in equilibrium with its decay products based on gamma ray measurements. The activity over the thyroid gland was measured and plotted at frequent intervals for several days. They found characteristic curves for various thyroid disorders. The curves for normal patients rose quite rapidly and then levelled off to a plateau in about 48 hours. The typical curve for thyrotoxic patients rose still more rapidly to reach a peak in about 6 hours. The radioactivity over the thyroid at that time was much greater than that of the normal subjects. The activity then

decreased rapidly to about the same level as that observed in the normals.

Hertz, Roberts and Salter (1942) studied the behavior of radioactive iodine in 22 cases of Graves' disease and in 2 normal individuals. The administered doses of iodine varied between 0.1 and 325 milligrams. Urinary excretion was measured in 12 thyrotoxic patients. The exact periods of urine collection are not given but they seem to have varied between 48 hours and several days. Four patients who received iodine for several days prior to the administration of the tracer dose excreted 19, 50, 64 and 87 percent of the tracer iodine. The excretion of radioactive iodine observed in eight uniodinized cases varied widely between 10 and 96 percent. These authors suggested that the wide variation in excretion of iodine was related to the rather large doses of carrier iodine used in some of these cases. With this thought in mind in a subsequent report Hertz and Roberts (1942) limited the amount of carrier iodine to less than 2 milligrams and observed a urinary excretion over a 72 hour period in 6 classic cases of Graves' disease to vary between 10 and 37 percent as opposed to an excretion of 62 percent in one normal subject.

Rawson, Evans, Means, Peacock, Lerman and Cortell (1944) studied the action of thiouracil upon the thyroid gland in Graves' disease. As a control they gave a tracer dose of radioactive iodine (total iodine 150 micrograms as sodium iodide) to an untreated case of Graves' disease and found that 13 percent of the dose was excreted in the urine during a five-day period.

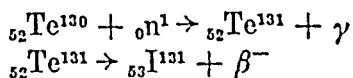
The quantity of iodine administered to patients has in earlier studies varied considerably and it has not only varied among different investigators but also in the same studies. It appears from the studies reported by Perkin, Brown and Lang (1934) and Hertz, Roberts, Means and Evans (1940) that the percentage of iodine collected by the thyroid varies inversely with the size of the dose. It seems quite likely then, that one would observe greater urinary excretion of iodine even in thyrotoxic patients when large doses of carrier iodine are given. As indicated above the minimal differences in the urinary excretion of radioactive iodine by normal subjects and by thyrotoxic patients observed by some investigators might well be explained by rather large amounts of carrier iodine administered with the tracers.

The first objective of this study was to determine the total urinary excretion of radioactive iodine in a 48 hour period in

various clinical states of thyroid function when the quantity of administered iodine was kept comparatively small and constant. The second objective was to determine the rate of urinary excretion of iodine in similar subjects. The third objective was to study the possibility of using these studies as a means of diagnosing certain diseases of the thyroid.

Methods.

$_{53}\text{I}^{131}$ which means iodine with the atomic number of 53 and the mass of 131 was used entirely. This isotope of iodine has a half life of 8 days and is therefore very convenient for biological experiments. It was supplied by Clinton laboratories Oak Ridge, Tenn., where it is produced in the chain reacting pile by the bombardment of metallic tellurium with slow neutrons according to the following nuclear reactions:



The isotope was delivered as carrierfree iodide, which means that all the iodine atoms in the solution are radioactive. The solution was contaminated with very minute amounts of tellurium, oxalates and sulfates. In this study no attempt was made to make a separation of the completely pure I^{131} , since the concentrations of the contaminants were too small to produce any biological effect. If not neutral on arrival the solution was neutralized with ammonia.

Dose of Radioactivity.

Some earlier investigators have given quite different amounts of radioactivity to various patients in the same study. It was felt safer to keep the amount of radioactivity constant. In deciding the dose of radioactivity to be administered one has to pay attention to the possibility of a radiation effect which can change the normal physiological processes. The dose used in this investigation has been 100 microcuries of I^{131} . This dose has been chosen mainly from a practical standpoint because it usually gives activity enough in the urine to make the urinary measurements quite simple. It is not known whether this dose will have any radiation effect over a long period of time, but I have fairly good evidence that this dose at least does not effect the urinary excre-

tion of iodine during the 48 hours the urine is collected. In a number of cases I have had the opportunity to compare the urinary excretion after a tracer dose with that after a therapeutic dose of I^{131} . The therapeutic dose has usually been about 100 times larger than the tracer dose. So far I have found very good agreement in the percentages excreted in the urine following tracer and therapeutic doses of I^{131} . Details of this study will be reported in a later communication. We must bear in mind, however, that a dose of 100 microcuries of I^{131} , calculated on an uptake of 30 percent of the dose in the thyroid and assuming that this amount of radioactivity stays there until it has decayed completely, will deliver a total dose of about 100—200 equivalent roentgens to the thyroid. Studies of possible radiation effects from these tracer doses are in progress by the author.

Standardization.

The standardization of I^{131} has been done according to the methods used at the Radioactivity Center of the Massachusetts Institute of Technology. It might be pointed out that the standardization in absolute units of various radioactive isotopes is a difficult physical problem. The methods for and the problems involved in the standardization of various isotopes of iodine will be discussed in a later report.

Carrier.

The quantity of sodium iodide acting as carrier for the radioactive iodine has been kept constant at 100 micrograms in all tests. The amount that should be used to make the test as sensitive as possible is not yet known. Theoretically the smallest amount of carrier, *i. e.* carrierfree radioactive iodine might give the highest sensitivity. My reason for not using carrierfree I^{131} has been that in some earlier experiments with carrierfree I^{131} the author observed a loss of activity on the walls of beakers and other glass containers. I therefore feared that when giving carrierfree I^{131} to patients unexpected losses could occur which might interfere with the results.

Procedure of the Radioactive Iodine Excretion Test.

The tracer dose of 100 microcuries of I^{131} with 100 micrograms of inert iodine (sodium iodide) as a carrier, was made up to 100

ml. One ml was taken off as a standard. The dose was given orally, usually in the morning before breakfast. Urine was collected in either of the following ways:

1. In one series of patients for 48 hours in two 24 hour specimen following the administration of the radioactive iodine.

2. In another series of patients for 48 hours in four specimens: 0—6 hours, 6—12 hours, 12—24 hours and 24—48 hours.

Instructions were carefully given to the patients concerning the collection of urine. If there was any doubt regarding the completeness of the urine collection, the test was discarded. The urine was collected in glass bottles and the urinary volumes were carefully measured.

Measurements of the Radioactivity.

0.20 ml of silver nitrate (1 mg Ag per ml) was placed on a silvered copper planchette. From each specimen of urine 0.2 ml was pipetted by means of sugar pipettes and mixed with the silver nitrate to get a precipitation of silver iodide. The samples were evaporated to dryness by lamp heaters. The samples were then measured with the common type of beta-ray Geiger-Müller counting tubes and the equipment used for measurement of radioactivity at the Massachusetts Institute of Technology. All measurements were done in duplicate. To get the percentage amount of radioactive iodine excreted in the urine the same procedure and measurements were made on the 1 ml aliquot taken from the tracer dose before the tracer dose was given. The accuracy of the method which is quite good, and the sources of error involved, will be discussed in a later publication.

Material.

Three groups of patients are included in this report. Group 1 comprises 25 cases of untreated thyrotoxicosis. None of these patients received iodine prior to the test. No selection of patients was made. They were taken as they appeared in the clinic. In none of the cases reported in this paper was there any doubt about the diagnosis, which was proved by the clinical and pathological findings as well as by their responses to treatment. Group 2 includes 6 patients with myxedema. All were classical cases in whom the diagnosis was well established. Group 3 consists of 15 euthyroid

Table 1.

Thyroid Status, Metabolic Rate and Radioactive Iodine Excretion Data: Thyrotoxicosis.

Case number	Size and type of goiter	Basal metabolic rate	Urinary excretion of radioactive iodine in percent		
			0—24 hrs	24—48 hrs	Total in 48 hrs
1	1½ × normal, diffuse	+47	12.1	1.5	13.6
2	5 × » »	+44	5.5	0.9	6.4
3	2½ × » »	+58	5.8	0.4	6.2
4	1 × » »	+21	21.8	0.8	22.6
5	3—4 × » »	+26	13.3	2.0	15.3
6	1½—2 × » »	+31	26.0	0.6	26.6
7	2—3 × » »	+66	15.1	0.7	15.8
8	1—1½ × » nodular	+49	21.9	2.5	24.4
9	2 × » diffuse	+34	13.5	3.2	16.7
10	3—4 × » »	+57	6.4	0.8	7.2
11	2—3 × » nodular	+20	23.3	0.6	23.9
12	1½ × » diffuse	+59	20.3	1.9	22.2
13	1 × » »	+30	10.3	0.8	11.1
14	2 × » »	+54	17.6	0.7	18.3
15	2½ × » »	+50	14.0	3.1	17.1
16	2½ × » nodular	+37	29.6	2.7	32.3
17	1 × » diffuse	+21	30.8	1.4	32.2
18	2 × » »	+34	23.0	1.9	24.9
19	1½ » » »	+50	13.0	2.7	15.7
20	2 × » » »	+55	22.7	2.6	25.3
21	2 × » » »	+21	8.3	1.2	9.5
22	2½ × » » »	+29	26.3	1.4	27.7
23	2½ × » » »	+18	21.7	3.3	25.0
24	3 × » » »	+48	20.5	2.8	23.3
25	2 × » » »	+46	13.4	3.4	16.8

(non-thyrotoxic) patients. These were patients having various non-metabolic diseases where it was reasonable to assume a normal thyroid function. This group of 15 patients was used as control material.

Results.

Table 1 presents the type and size of goiter, the basal metabolic rate, and the urinary excretion data for the 25 cases of thyrotoxicosis. The mean excretion of I¹³¹ in 48 hours is 19.2 percent with variations between 6.2 percent and 32.3 percent. Table 2 and 3 give the basal metabolic rates and urinary excretion data for the 6 cases of myxedema and the 15 euthyroid subjects. The excretion in the myxedematous patients varied between 72.4 percent and 91.7 percent. The mean excretion for the controls was 66.6 percent

Table 2.

*Basal Metabolic Rate and Radioactive Iodine
Excretion Data: Myxedema.*

Case number	Basal metabolic rate	Urinary excretion of radioactive iodine in percent		
		0—24 hrs	24—48 hrs	Total in 48 hrs
26	—30	61.0	21.8	82.8
27	—26	66.6	20.2	86.8
28	—44	65.0	24.3	89.3
29	—31	59.2	16.3	75.5
30	—42	57.2	15.2	72.4
31	—36	76.0	15.7	91.7

Table 3.

*Basal Metabolic Rate and Radioactive Iodine
Excretion Data: Euthyroid Subjects.*

Case number	Basal metabolic rate	Urinary excretion of radioactive iodine in percent		
		0—24 hrs	24—48 hrs	Total in 48 hrs
32	—1	60.2	7.9	68.1
33	—4	67.7	3.0	70.7
34	+2	54.5	10.4	64.9
35	—7	55.7	1.8	57.5
36	+3	61.7	1.6	63.3
37	+6	56.0	1.8	57.8
38	+6	48.3	4.4	52.7
39	—9	56.6	12.4	69.0
40	+11	69.3	14.8	84.1
41	—	57.8	3.9	61.7
42	—	69.3	6.8	76.1
43	—3	64.7	8.0	72.7
44	—	71.5	6.2	77.7
45	—	52.7	7.3	60.0
46	—	58.2	3.8	62.0

with the range of 52.7 percent to 84.1 percent. Fig. 1 presents a diagram of the 48 hour urinary excretion in these three groups studied.

Discussion.

The urinary excretion of radioactive iodine in this group of thyrotoxic patients is considerably less than in the group of euthyroid subjects. The variation in the control group seems

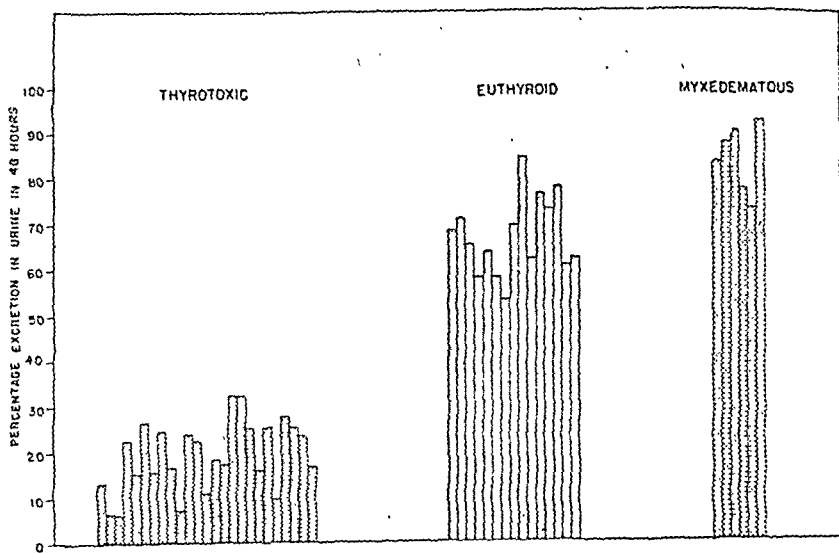


Fig. 1. The 48 hour urinary excretion of radioactive iodine in the thyrotoxic, the euthyroid and the myxedematous groups.

somewhat disturbing but is probably not greater than can be expected in any biological test like this. The variation in the thyrotoxic group is not surprising since the severity of the disease as well as the size of the thyroid varies considerably. The basal metabolic rates ranged between plus 18 and plus 66 percent and the thyroid size as judged by palpation varied between normal and five times normal size. It may be pointed out, however, that so far no overlapping has been observed between the two groups. The highest excretion for a thyrotoxic patient is 32.2 percent and the lowest for a euthyroid is 52.7 percent. There is, however, always a good possibility that such overlapping will be observed when more studies are done.

In fig. 2 is plotted the urinary excretion versus the basal metabolic rate for the thyrotoxic group. There seems to be only a very small, if any, correlation between these two tests. This must be interpreted as indicating that the two tests do not measure the same function of the thyroid gland. The basal metabolic rate is by no means a specific test of thyroid function. It can be used, however, as an index of the organism's response to thyroid hormone. Since the test with the radioactive iodine is a measurement of the thyroid gland's avidity for iodine they do not necessarily run parallel. Which test has more specific validity cannot be judged with the information available at the present time. The

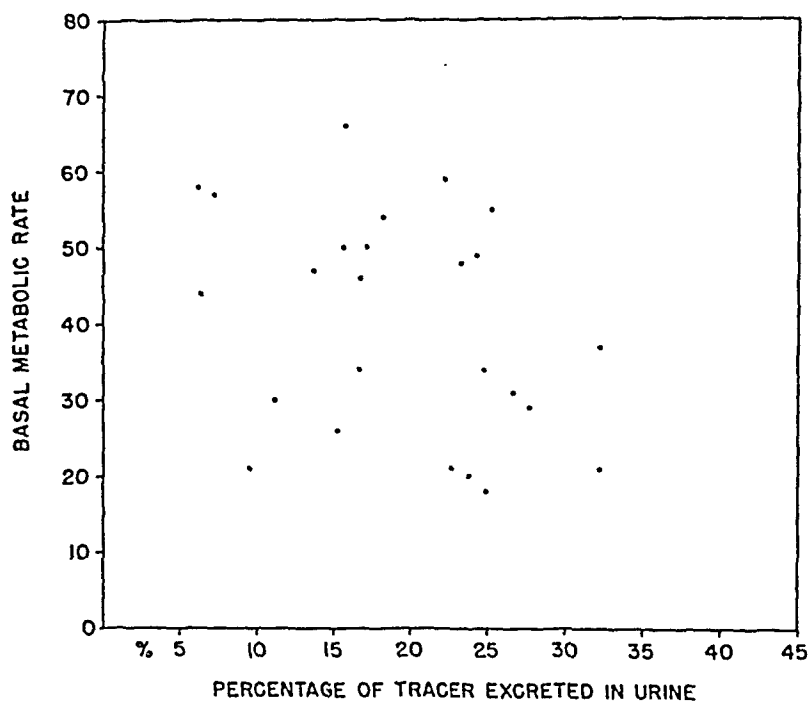


Fig. 2. Comparison between basal metabolic rate and radioactive iodine excretion in thyrotoxic patients.

author has made observations in a few cases of early thyrotoxicosis which may suggest that the increased avidity of the gland for iodine is an earlier appearing disturbance than the increase in basal metabolic rate.

The 48 hour excretion of radioactive iodine in the 6 cases of myxedema falls very much in the same range as the controls. If one, however, divides the total 48 hour excretion in the 0—24 hour period and the 24—48 hour period, greater differences are found (see fig. 3). The diagram demonstrates that in euthyroid subjects the excretion in the 24—48 hour period averages 6.3 percent with the highest value 14.8 percent, whereas in patients having myxedema the lowest value is 15.2 percent. The number of cases is too small to permit drawing any definite conclusions. The figures indicate, however, as would be expected, that the urinary excretion is slower in myxedema than in euthyroid patients. This is also supported by similar findings in two cases of myxedema studied by Hamilton and Soley (1939).

These differences in the rates of excretion in euthyroidism and myxedema led me to investigate the possibility of demonstrating a dynamic difference between the hyperthyroid group and the

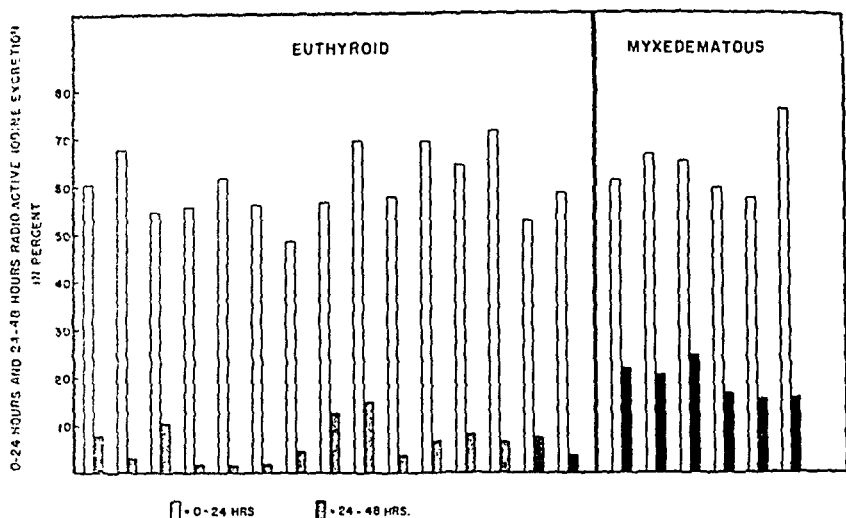


Fig. 3. 0-24 hours and 24-48 hours urinary excretion of radioactive iodine in euthyroid and myxedematous patients.

euthyroid group by measuring the rate of excretion at shorter time intervals. Studies in which the urine was collected in 0-6, 6-12, 12-24 and 24-48 hour specimens were done in 8 euthyroid subjects and in 10 thyrotoxic patients. The urinary excretion curves for these two groups are recorded graphically in fig. 4. The curves in both groups seem to be exponential. In the thyrotoxic group there is an initial phase of about 6 hours of relatively rapid excretion after which time the excretion becomes comparatively slow. There is, however, some excretion going on during the entire 48 hours of observation. In the non-thyrotoxic group there is also from the beginning a rapid phase, probably more rapid than in the thyrotoxic group. Furthermore, this rapid phase is going on for a longer period of time, about 12-24 hours, before the curve levels off. It is reasonable to assume that the striking differences in the urinary excretion curves in the two groups are due to the differences in avidity for iodine of the thyroid gland in these two clinical states. There are no reasons to believe that the real renal excretion rate should be slower in the thyrotoxic group, since no kidney damage has been demonstrated in these patients. It is possible that a mathematical and statistical analysis of the curves presented will be helpful. Such a study is in progress.

The author is well aware that the urinary excretion of radioactive iodine is only an indirect measurement of thyroid function. However, there are good reasons to assume that practically all

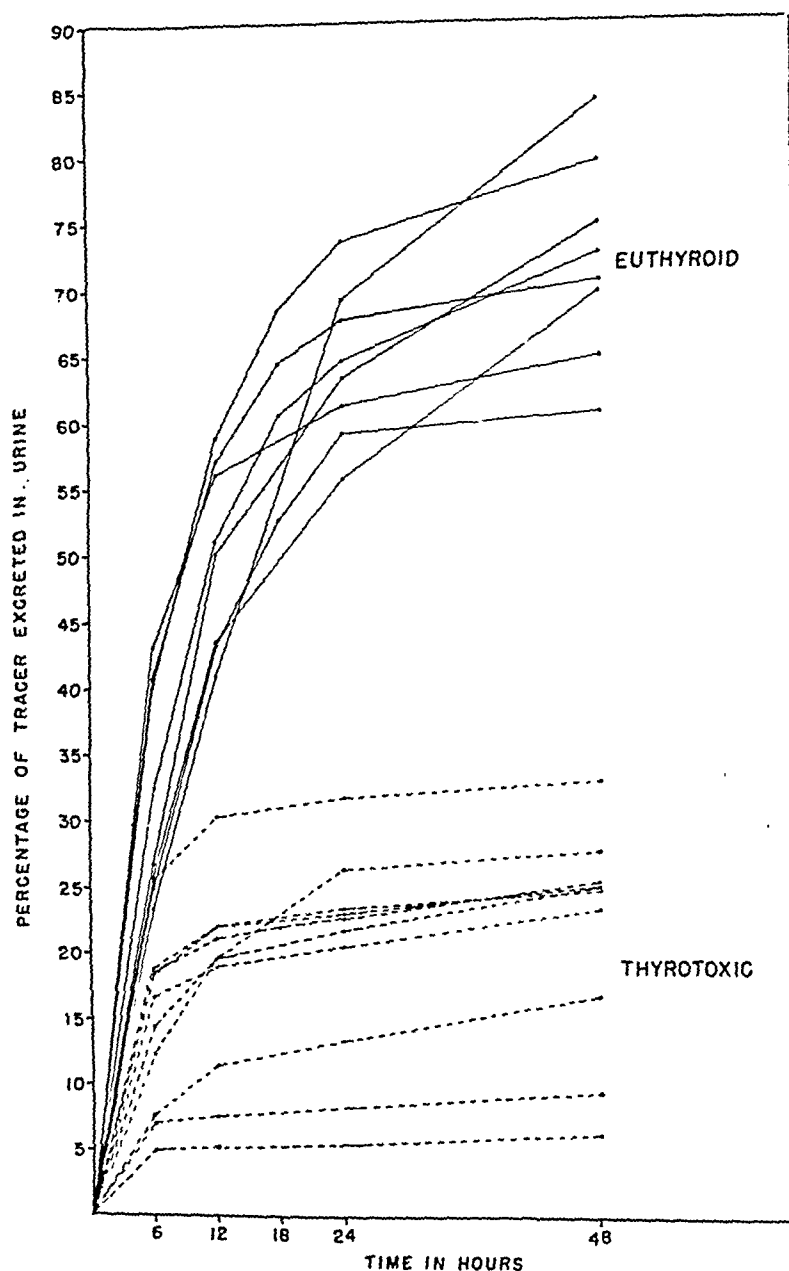


Fig. 4. Urinary excretion curves for euthyroid and thyrotoxic patients.

of the radioactive iodine in a tracer dose is either collected by the thyroid or excreted in the urine. The fecal excretion is very low as was first demonstrated by Hamilton and Soley (1939). By measuring the urinary excretion and the amount of radioactive iodine in the thyroidectomized gland after preparation with

thiouracil Rawson et al. (1944) were able to account for 85 to 100 percent of the administered radioactive iodine. Thus the reasons for following the urinary excretion are very good. Furthermore, the analysis for radioactivity in urine is technically a simple procedure.

A method of measuring radioactive iodine in the thyroid with in vivo methods would be a more direct approach to the study of iodine collection by the thyroid. Such studies were carried out by Hamilton and Soley (1940). Unfortunately the present methods for such measurements are very inaccurate. Attempts to improve this technique are in progress in this laboratory.

The excretion of radioactive iodine, which, as indicated above, is believed to reflect the collection of iodine by the thyroid is influenced by a variety of factors. No doubt the functional state of the thyroid gland plays a very important rôle in determining the results of such studies. The observation that the urinary excretion of radioactive iodine by the 25 thyrotoxic patients reported in this study was considerably less than that excreted by euthyroid subjects would indicate that the thyroids of such patients, which are characteristically hyperplastic and lacking in iodine have a much greater avidity for iodine than the normal involuted thyroid. This thesis is supported by the observation of Keating, Rawson, Peacock and Evans (1945) who observed that the thyroids of chicks made hyperplastic by previous treatment with thyrotropic hormone collected considerably more radioactive iodine than did their untreated controls. It should be pointed out, however, that Rawson et al. (1944) have demonstrated that the hyperplastic thyroids of thyrotoxicosis though lacking in iodine do under the influence of thiouracil collect very little radioactive iodine. This is no doubt due to one of the pharmacologic actions of thiouracil.

From the earlier studies by Hamilton and Soley (1939, 1940) one might suspect that iodine in large doses interferes with the collection of tracer iodine through some of its pharmacological actions. They administered tracer amounts of radioactive iodine with as much as 14 milligrams of carrier iodine and observed no difference in the excretion of the labelled iodine by thyrotoxic patients as compared to normal subjects. Another explanation for their failure to demonstrate any difference between the two groups could also be made on the basis of a dilution of the tracer with the larger doses of inactive iodine. This thesis is supported by the observations of Hertz, Roberts, Means and Evans (1940)

that the percentage uptake of radioactive iodine by the thyroids of rabbits varied inversely with the dose of carrier iodine.

It might be pointed out that in general a tracer is supposed to label a specific quantity of an element or a substance and in this way make it recognizable from other material. In biological studies tracers may be used in labelling naturally occurring substances without increasing the amount of that substance above a physiological level. To be more specific as soon as an administered mixture of radioactive and carrier iodide reaches the blood it is mixed with the iodide already circulating in the plasma. Thus the tracer iodine acts to label the entire circulating iodide of the blood and will measure the percentage quantity of the total circulating blood iodide which is collected by the thyroid or excreted by the kidney. The amount of normal circulating iodide in the plasma is of the magnitude of 1—2 micrograms per 100 ml.

What quantity of iodine should then be administered? The 8 day isotope of iodine which has been used in this study is delivered carrierfree. That means that the quantity of iodine in a 100 microcurie dose is of the magnitude of 0.001 micrograms and therefore can be neglected. Theoretically such a minute quantity would be desirable because in such a case the radioactive iodine is really acting as a tracer for the iodine already present in the blood. This has so far been avoided because of the fear that losses of such infinitesimal quantities can occur on the walls of containers or in the gastrointestinal wall or by other means. On the basis of certain animal experiments in which studies with varying amounts of carrier iodine were done, the dose of 100 micrograms of carrier iodide was selected for these clinical studies. Such an amount would probably increase the blood iodide concentration by about 1 microgram per 100 ml plasma. I doubt that this increase in the blood iodide concentration will change the iodine metabolism so as to effect the result of the test.

There are probably certain extrathyroidal factors which influence the results obtained in such urinary excretion studies. Among these should be considered the rate of absorption from the gastrointestinal tract. Hamilton (1938) demonstrated that most of the iodine was absorbed in one hour and that the absorption was complete in 3 hours. It is possible that the presence of food in the stomach interferes with the rate of absorption. For this reason with few exceptions the tracers have been administered to patients when fasting.

It has been pointed out by Salter (1940) that though the mechanisms by which the kidney excretes iodine are not known, certain diseased kidneys have a decreased urinary excretion of iodine.

It is quite likely that in congestive heart failure the renal excretion of iodine is impaired. Though this is not well established, Rowntree, Fitz and Geraghty (1913) in a study on dogs found that the excretion of iodide was prolonged in chronic passive congestion of the kidney.

In all work with radioactive isotopes one has to pay attention to the possibility of radiation effects. The maximum permissible dose is 0.1 roentgen per day for total body radiation from radium or roentgen rays. In this study 100 microcuries of I^{131} has been used. Assuming that the radioactive iodine taken up by the thyroid, stays there until it has disintegrated, this dose will give a total dose of 100—200 equivalent roentgens in about 7 weeks and by far exceed the maximum permissible dose of 0.1 roentgen per day. But it must be pointed out that this 0.1 roentgen per day is a dose that is considered to be harmless for an indefinite period of radiation. In order to get down to the level of 0.1 equivalent roentgens a day one would have to use doses of about 1 microcurie of I^{131} . This unfortunately is not practicable to use with the present methods of measurement. That the doses employed in this investigation do not cause any disturbance of the urinary excretion in the 48 hours under study has been demonstrated by the author in a number of patients where a comparison has been made between the urinary excretion curves for a tracer dose and for a therapeutic dose of radioactive iodine. The agreement in urinary excretion in these cases has been very good.

In order to get more information on the biological effects of the radiation of radioactive iodine a study on animals is in progress. One of the objectives is to define the doses that can safely be employed both in short and long term experiments. Without such knowledge one has always to consider the possibility that the radiation from a tracer dose may produce biological effects on the physiology under study. It may again be emphasized that the radiation has not effected the results presented here.

The fact that the hyperplastic gland in thyrotoxicosis has an increased avidity for iodine has long been known, but the introduction of radioactive iodine has given us a tool to measure this increased avidity on quantitative basis. It is possible that this phenomenon can be utilized for diagnostic purposes. The results

so far are encouraging. The clinical diagnosis of thyrotoxicosis is in most cases easy. There are, however, a certain number of borderline cases and of masked hyperthyroidism which are very difficult to evaluate by the clinical findings and the laboratory methods available. There seems, therefore, to be a need for methods to facilitate this differential diagnosis. The results presented in this report suggest that the urinary excretion of radioactive iodine after a tracer dose can be used for this purpose and an investigation of the clinical value and limitations of this new iodine tolerance test is in progress by the author.

Summary.

1. The use of radioactive iodine in the study of disturbances of the thyroid function is discussed.

2. Tracer doses of radioactive iodine have been orally administered to three groups of patients: thyrotoxic, euthyroid and myxedematous. The urinary excretion of the radioactive iodine has been measured.

3. The 48 hour urinary excretion for 25 patients with thyrotoxicosis varied between 6.2 percent and 32.3 percent with an average of 19.2 percent.

4. The 48 hour urinary excretion for 15 euthyroid patients (controls) ranged between 52.7 and 84.1 percent with an average of 66.6 percent.

5. The 48 hour urinary excretion in 6 cases of myxedema varied between 72.4 and 91.7 percent.

6. The urinary excretion of radioactive iodine in the group of thyrotoxic patients is considerably less than in the group of euthyroid patients. No overlapping between the two groups has been observed so far.

7. Thyroidal and extrathyroidal factors which influence the urinary excretion of radioactive iodine have been discussed.

8. It is possible that the urinary excretion of radioactive iodine after a tracer dose can be used for diagnostic purposes. An investigation of the clinical value and limitations of this new iodine tolerance test is in progress.

Grateful acknowledgement is made to Prof. J. H. Means and Prof. R. D. Evans for the facilities provided in their laboratories

for this work and to Dr. R. W. Rawson and Dr. Rex G. Fluharty for their helpful criticism of this work and in the preparation of this paper.

Bibliography.

Baumann, E.: *Zeitschrift f. Physiol. Chemie*, 22, 1, 1896. — Elmer, A.: Oxford University Press, London, 1938. — Fermi, E.: *Nature*, 133, 757, 1934. — Hamilton, J. G.: *Am. J. Physiol.*, 124, 667, 1938. — Hamilton, J. G. and Soley, M. H.: *Am. J. Physiol.* 127, 557, 1939. — Hamilton, J. G. and Soley, M. H.: *Am. J. Physiol.* 131, 135, 1940. — Hertz, S., Roberts, A. and Evans, R. D.: *Proc. Soc. Exp. Biol. and Med.* 38, 510, 1938. — Hertz, S., Roberts, A., Means, J. H. and Evans, R. D.: *Am. J. Physiol.* 128, 568, 1940. — Hertz, S., Roberts, A. and Salter, W. T.: *J. Clin. Investigation*, 21, 25, 1942. — Hertz, S. and Roberts, A.: *J. Clin. Investigation*, 21, 31, 1942. — Hevesy, G.: *Biochem. J.*, 17, 439, 1923. — Joliot, F. and Curie, I.: *Nature*, 133, 201, 1934. — Keating Jr., F. R., Rawson, R. W., Peacock, W. and Evans, R. D.: *Endocrinology*, 36, 137, 1945. — Marine, D. and Feiss, H. O., *J. Pharmacol. and Exper. Therap.*, 7, 557, 1915. — Marine, D. and Rogoff, J. M.: *J. Pharmacol. and Exper. Therap.* 8, 439, 1916. — Perkin, H. J., Brown, B. R. and Lang, J.: *Canad. M. A. J.*, 31, 365, 1934. — Rawson, R. W., Hertz, S. and Means, J. H.: *Ann. Int. Med.* 19, 829, 1943. — Rawson, R. W., Evans, R. D., Means, J. H. Peacock, W. C., Lerman, J. D. and Cortell, R. E.: *J. Clin. Endocrinology* 4, 1, 1944. — Rawson, R. W. and McArthur, J.: *J. Clin. Endocrinology*, 7, 235, 1947. — Rowntree, L. G., Fitz, R. and Geraghty, J. T.: *Archives of Int. Medicine*, 11, 120, 1913. — Salter, W. T., Harvard University Press, Cambridge, Mass.: 1940. — Watson, E. M.: *Endocrinology*, 20, 358, 1936. — Watson, E. M.: *Endocrinology* 22, 528, 1938.

From The Jewish Hospital, Alexandria, Egypt.

The Pellagra-Electrocardiogram and its Significance.

By

F. MAINZER,

Consultant Physician to the Hospital.

(Submitted for publication August 27, 1947.)

In spite of a dissenting paper by Porter & Higginbotham (18) reproduced in 1941 in Harris & Harris "Clinical Pellagra" (9), it has been definitely shown that electrocardiographic alterations are encountered in a number of pellagrins. Feil (3) was the first to note this observation and his findings were confirmed by Mainzer & Krause (17); furthermore we observed the disappearance of the cardiographic changes during treatment with nicotinic acid, a fact confirmed by Rachmilevitz & Braun (19, 20).

There is nothing characteristic for pellagra in these changes, since similar tracings are encountered in other diseases, especially in coronary sclerosis; indeed this very fact, stressed in our earlier paper, induced the erroneous statements of Porter & Higginbotham (18). Hence a single abnormal tracing taken in a pellagrin cannot be definitely proved to be brought about by pellagra, although in young patients the causal relation is probable by exclusion of other causes. In most cases there is, however, a general parallelism between the development of the cardiographic tracings, if taken in series, and the other pellagrous phenomena, substantiating this causal relation; a further argument is the quick disappearance of the cardiographic alterations of pellagrins treated with nicotinic acid [Mainzer & Krause (17), Rachmilevitz & Braun (19, 20)], as observed in some instances.

However, not all the cases are conclusive; as it will be shown in this study, the cardiogram remains abnormal in a considerable number of pellagrins, in spite of nicotinic acid in sufficient dosage; in other cases the normalisation proceeds slowly and incompletely.

Moreover, the identification of nicotinic acid deficiency and pellagra is far from being justified; pellagra, as defined by Sydenstricker (26), is "a B-group avitaminosis in which lack of nicotinic acid is predominant but in which other vitamins are depleted to a level of physiologic inadequacy"; actually the concurrent deficiency of thiamin [Weiss & Wilkins (29) and others], Riboflavin [Sydenstricker (26)], folic acid [Spies (22)] and cholin [Th. Gillman & J. Gillman (7), Mc Henry & Patterson (14)], has been shown in many cases of pellagra.

The cardiographic changes produced by thiamin deficiency are well known [Weiss & Wilkins (29), Weiss, Haynes & Zoll (30)]. With the exception of nicotinic acid there is no similar evidence for the other B-factors; even the effect of nicotinic acid itself is incompletely understood and partially controversial.

Hence a further study of the cardiographic changes in pellagrins seemed warranted.

Cardiographic Findings.

The present paper is based on the study of 45 pellagrins with 139 tracings.

Table I summarizes the frequency of the different pellagra manifestations in these cases.

Table I.

Frequency of the more important pellagra manifestations.

1	2	3	4	5		6	7	8	Outcome	
Total number of pellagrins	Skin	Mouth	Diar-rhoea	Anemia		Psychic disturbances	Pellagra-cardiogram	Insulin hypersensitivity	Cured or improved	dead
				macro-cytic	micro-cytic					
45	37	40	30	2	14	22	38	23 ¹	36	9 ²

¹ Not investigated in 14 cases; inconclusive in 2 diabetic pellagrins; no hypersensitivity in 6 cases.

² 2 above 70 years.

For technical reasons we recorded only the manifestations present during the time of clinical observation without reference to those reported in the past history.

Column 8 of the table, headed "Insulin Hypersensitivity" is referring to the following fact: in pellagrins, a small dose of insulin (5 units) given by subcutaneous injection induces with few exceptions an abnormally great decrease of the blood-sugar level, often occurring with severe symptoms of hypoglycemia [Mainzer (15, 16)]; in healthy people under these conditions the blood-sugar decreases not below a level of 60 mg% (procedure of Hagedorn-Jensen); in pellagrins we found levels as low as 18 mg%. The theoretical significance of this phenomenon is beyond the scope of this paper; its practical importance, however, derives from the fact that the reaction remains mostly unchanged, even in clinically cured pellagra.

Table I shows that "pellagra-cardiograms" are as frequent in this disease as the cutaneous phenomena and the abnormal insulin-hypoglycemia; only one out of five patients has a tracing without indications of pellagra-influence; even intestinal symptoms are less frequent.

The shape of the cardiographic changes has been previously discussed [Feil (3), Mainzer & Krause (17), Rachmilevitz & Braun (19, 20)]. Most frequent are the abnormalities of the final wave; it is flat, iso-electric or negative [in some cases very large according to Feil (17)]. This author observed in five cases an S-T interval of the Pardee-type; deviation of the S-T interval from the iso-electric level is not infrequent.

In serious cases the voltage of the ventricular complex is diminished. Notching and slurring of the ventricular complex is also encountered.

In Table II the cardiographic findings in pellagrins are summarized.

The five cases of column 3 require a separate discussion. These are borderline-cases not to be classified as abnormal, so much more with reference to the great variation of the normal cardiogram as shown lately by Stewart & Manning (23) and Graybiel, McFarland, Gates & Webster (8). However, a series of tracings taken during treatment showed a continuously increasing voltage of the T waves together with a more typical shaping of the S-T intervals. So the pellagra-influence, on apparently "normal" tracings, became manifest.

Table II.

Electrocardiographic changes in pellagrins.

1	2	3	4	5	6	7
Number of pellagrins	Ecg normal and unchang- ed	Ecg border- line trac- ings with improve- ment	Ecg abnor- mal with improve- ment	Ecg abnor- mal be- coming worse	Ecg abnor- mal un- changed	Ecg abnormal with con- current coronary insufficiency
45						
a) several tracings	3	5	15	5	2	2
b) one tracing only	4	—	—	—	8	1
c) Total	7	5	15	5	10	3
	normal ecgs 12		abnormal pellagra ecgs 30			
	pellagra ecgs 35					

By including these "normal" tracings the total frequency of pellagra-cardiograms increases considerably.

In our previous study, differentiating only between normal and pathological tracings, we found 43 % of the cardiograms not influenced by the disease; Feil (3) found 50 %, Rachmilevitz & Braun (20) 33 %; now by including the "normal" pellagra-cardiograms our rate is only 25 %.

The relation between the development of the cardiographic alterations and the clinical course of the pellagra under treatment (especially with nicotinic acid) form a basis for pathogenetic interpretation.

According to Rachmilevitz & Braun this relation is very simple. In their cases the initially pathological pellagra-cardiograms became normalised after a few (4—5) days of nicotinic acid-treatment; only in rare instances the required time was longer. So they felt, that the abnormal tracings were brought about by a disturbance of the myocardial metabolism induced by niacin-deficiency. The retardation of the niacin-effect on the cardiogram was supposed to be due to an insufficient utilization of the vitamin produced by a disturbed liver function, as indicated by hypoproteinemia.

The results of the present study are only in partial agreement with these statements and cannot be framed within the pathogenetic interpretation of the authors.

Table III.

Age and sex-distribution of pellagrins.

Years	1	2	3	4	5	6	7	8
	11—20	21—30	31—40	41—50	51—60	61—70	above 70	Total
Males	—	—	5	4	6	5	3	23
Females	1	6	2	4	6	3	—	22
Total	1	6	7	8	12	8	3	45

Table IV.

Distribution of pellagra-electrocardiograms according to age and sex.

	Below 50 years	Above 50 years	Total
Males	7	10	17
Females	11	7	18
Total	18	17	35

Our observations are summarized in the tables III—VII.

The dosage of the pellagra-curative substances, as referred to in the tables, was as follows (per day):

Yeast: 30—100 g of fresh yeast,

Liver extract: 2 ml of various preparations declared as the equivalent of 500 g fresh liver (before the standardisation in units), subcutaneously injected,

Thiamin: 10—20 mg given by subcutaneous injection,

Nicotinic-acid: 500 mg, of which half the amount by mouth, half by intramuscular injection; in some cases this amount was reduced in the course of treatment to 300 mg.

Some observations belong to the pre-nicotinic-acid-period; these cases were treated with a high-caloric diet, yeast, liver extract and sometimes thiamin.

The cardiograms were taken in most cases every five to ten days in the beginning, with greater intervals during the later stages.

By taking series of tracings during the course of the disease all cardiograms suspected for coronary insufficiency could be examined for the influence of pellagra; in two pellagrins the combined influence of the two diseases could be substantiated by this procedure.

c) Analysis of the substances dialyzed from the blood into the salt solution is rendered difficult. d) It is difficult to obtain sufficient quantities of colloids; colloids with a satisfactory degree of purity would be far too expensive in practice.

The above also applies essentially to polyvinyl-alcohol.

The present apparatus affords the possibility to maintain the fluid balance by means of hydrostatic pressure. As it is possible even to obtain a stronger dehydration in the modified form of the apparatus already mentioned by one of us (A. 1947), we have no »colloid» problem. We have therefore discontinued to work on it.

2. Hydrostatic Pressure.

The cellophane casing of the apparatus contains a limited and definite amount of blood, which is practically independent of the pressure in the casing. By suitably adjusting the pressure it is possible to maintain equilibrium between the blood flowing through the casing, and the surrounding salt solution. We have studied the fluid balance both in vivo and in vitro.

In vitro: The fluid balance between heparinized animal plasma and a salt solution has been studied in the following manner.

A relatively small dialysis apparatus was used for the experiments. The plasma was made to flow from a container placed about 1.5 m above the level of the apparatus. The rate of flow was controlled by a valve described in an earlier publication (A., 1947). All conditions were constant except for the fact that the height of the rubber tube, through which the fluid left the apparatus (= the hydrostatic pressure) was changed. From this outlet tube the plasma was allowed to flow into a vessel, whence it was poured back into the first mentioned high container. In this manner about 200—250 ml. plasma circulated through the apparatus at a rate of $3/4$ —1 litre per hour during six hours. The experiments were carried out at room temperature.

Table 1 shows *firstly* the length of the outlet tube (= the hydrostatic pressure exercised against the contents of the casing during the major part of the experiment) and *secondly* the change of the protein content of the plasma during 6 hours' dialysis as determined according to Van Slykes copper sulphate method.

As was to be expected, we found that at the low hydrostatic pressures, the protein content of the plasma fell — *i. e.*, fluid flowed from the salt solution to the plasma — whilst the protein content remained constant or increased a little with the higher pressures.

Table VI.

Interrelations between the electrocardiographic development and the course of the pellagra.

Pellagra and cardiogram both improved	Pellagra and cardiogram both worse	Pellagra improved, cardiogram worse	Pellagra worse, cardiogram improved	Pellagra improved or worse, cardiogram unchanged	Total
1	2	3	4	5	6
19	5	1	2 ¹	5	32

Table VII.

Time required for the maximal improvement of the cardiogram in pellagrins treated with nicotinic-acid.²

Maximal Improvement after days:					
1—10	11—20	21—30	More than 30	Inconclusive	Total
1	2	3	4	5	6
6	3	3	1	7	20

In one case, a cardiogram incompletely normalised during niacin-treatment became perfectly normal after additional treatment with yeast; in another cases the same observation was noted with thiamin.

Another group of pellagra-tracings remained completely unchanged during many weeks of treatment.

The same cardiographic developments were noted with combined niacin-yeast treatment; there was complete or incomplete normalisation or no effect on the tracing.

In the whole, as stated, the development of the cardiogram corresponds to the general course of the illness; however some exceptions were noted. Twice there was a continuous improvement of the tracing despite of worsening pellagra, even in one case with fatal issue. Once the cardiogram became much worse in a pellagrin cured clinically.

The interrelations are summarized in table VI.

Table VI shows that in a quarter of the cases, the expected parallelism is absent.

¹ 1 fatal case.

² Cases with simultaneous treatment with yeast, thiamin or liver extract are included.

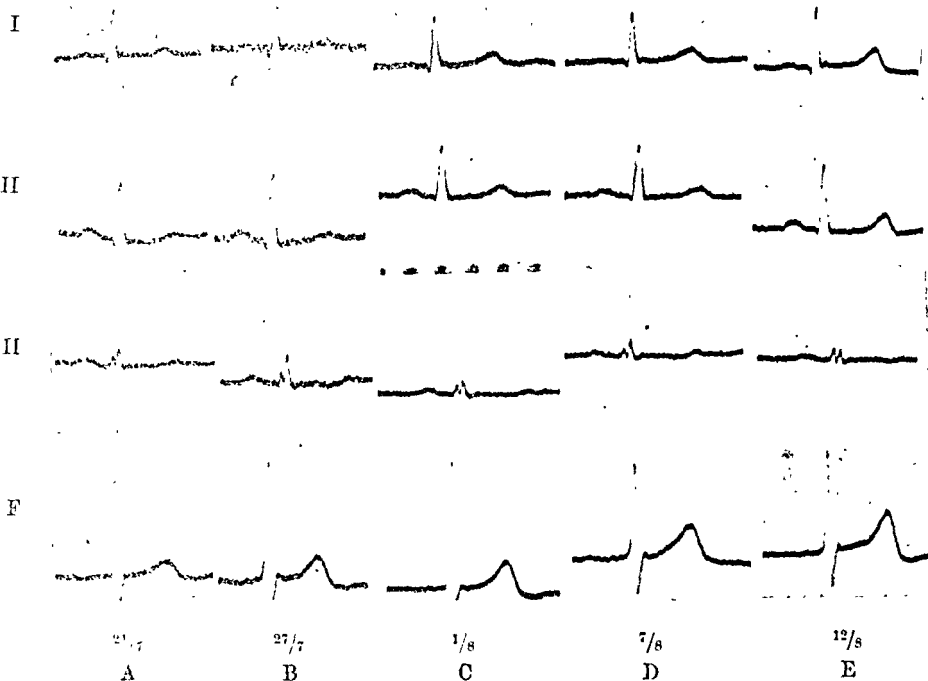


Figure I. Slowly progressing normalisation of a pellagra electrocardiogram.

Table VII summarizes the time-relations between niacin-treatment and the improvement of the cardiogram, including the cases with incomplete normalisation. The table shows that a quick improvement of the cardiogram is often observed. Cases with delayed normalisation are equally frequent.

Some of the findings above related will be illustrated by examples.

Figure I illustrates a very slow normalisation of a cardiogram during niacin-treatment.

Case 1: The patient, a night-porter, aged 60, was unemployed and without resources for 4 months; his meals consisted mainly of bread and dry beans. Owing to his insufficient nutrition, he felt weak. Ten days before admission to the hospital (21/7/39), he observed a redness of the skin of both hands.

The examination revealed a fresh dermatitis on the dorsum of both hands, glossitis marginalis with erosions, atrophy of the lingual papillae, psychic disturbances, especially a silly euphoria; there were no other pellagra manifestations, no diarrhoea, no anemia.

The insulin-test showed a pronounced hypersensibility, disappearing during treatment (a rather exceptional development, mostly seen in fresh cases).

The minimal blood-sugar levels after 5 units insulin were as follows (procedure- Hagedorn-Jensen):

Date	Minimal blood-sugar level	
	mg.	%
22/7/39	46	
29/7/39	18 (!)	
5/8/39	43	
11/8/39	73	

The patient was treated with nicotinic acid, 0.25 g per day injected and the same amount by mouth.

After a fortnight all pellagra symptoms had disappeared.

Electrocardiograms.

Time measurements.

Denomination	Date	Pulse rate	P-R	R-S (in seconds)	R-T
A	21/7/39	92	0.17	0.07	0.35
B	27/7/39	84	0.17	0.07	0.35
C	1/8/39	68	0.17	0.07	0.40
D	7/8/39	72	0.17	0.07	0.38
E	12/8/39	56	0.17	0.07	0.38

The initial cardiogram gives just a hint of positive T waves in the limb and has a flat T in lead IV F. The subsequent tracings show a slowly progressing normalisation; even after two weeks T_2 and T_3 remain flat; after three weeks they are normal and no further change occurs.

During a second hospital stay, the initial cardiogram was similar to figure I D and after one month's treatment to figure I E.

Figure II shows the normalisation of a borderline-cardiogram in pellagra.

Case 2: A woman, aged 30, could not regain her previous health after the birth of her third child. She felt weak; from time to time she suffered from diarrhoea without pain. The menstruation had not reappeared after weaning. Eight days before entering the hospital (30/7/40), she observed a redness of the backs of both hands. The husband was unemployed for the last six months and the nutrition of the whole family was insufficient.

There were profound alterations of the skin on face, neck, both hands and arms as well as both legs and feet; partly the lesions were fresh with erythema, partly older with hyperkeratosis and ulceration. Glossitis marginalis and atrophy of the papillae of the tongue were present. Actually, there was no diarrhoea and the psychic behaviour was normal.

The insulin-test revealed a marked-hypersensitivity, remaining unchanged after clinical cure of the pellagra.

Minimal blood-sugar levels after 5 units insulin.

Date	Blood-sugar level	
	mg	%
1/8/40	32	
12/8/40	27	
23/8/40	33	

At the beginning the patient was treated with 0.50 g nicotinic acid per day, of which half the amount by injection; later the nicotinic acid given by mouth was replaced by 100 g of fresh yeast. The skin alterations disappeared quickly and at the time of dismissal (25/8/40) only a slight desquamation remained.

Electrocardiograms.

Time measurements.

Denomination	Date	Pulse rate	P-R (in seconds)	R-S (in seconds)	S-T
A	31/7/40	90	0.17	0.07	0.32
B	1/8/40	69	0.18	0.07	0.39
C	5/8/40	53	0.18	0.07	0.40
D	21/8/40	60	0.18	0.07	0.40

In Figure II A the T waves are flat in all leads, but cannot be classified as definitely abnormal ($T_1 = 1$ mm, $T_2 = 1.5$ mm). The subsequent development, however, showed that they were pathological; in fact, during the treatment with nicotinic acid, the T waves increased at the beginning, but later no further change occurred with niacin alone (Figure II B and Figure II C — 12/8); following an additional treatment with yeast for 9 days the cardiogram became typical by further increase of the T waves (Figure II D: $T_1 = 4.0$ mm, $T_2 = 3.5$ mm).

The figure III shows a slow improvement of a pellagra tracing, remaining incomplete in spite of continued treatment with niacin, thiamin and extract of liver and stomach.

Case 3: A merchant, aged 36, without contributory past history, especially without rheumatic disease, suffered three years ago from stomach pain, considered as peptic ulcer and treated with alkaline powders.

Six months before admission without extrinsic cause and while fed with an unrestricted diet, a painless diarrhoea appeared, slowly increasing; at the beginning he had 3—4 half-formed stools, later 4—6 completely liquid evacuations. He lost 10 kgs and felt increasingly weak. In the last time he became irritable, quarrelsome and preoccupied.

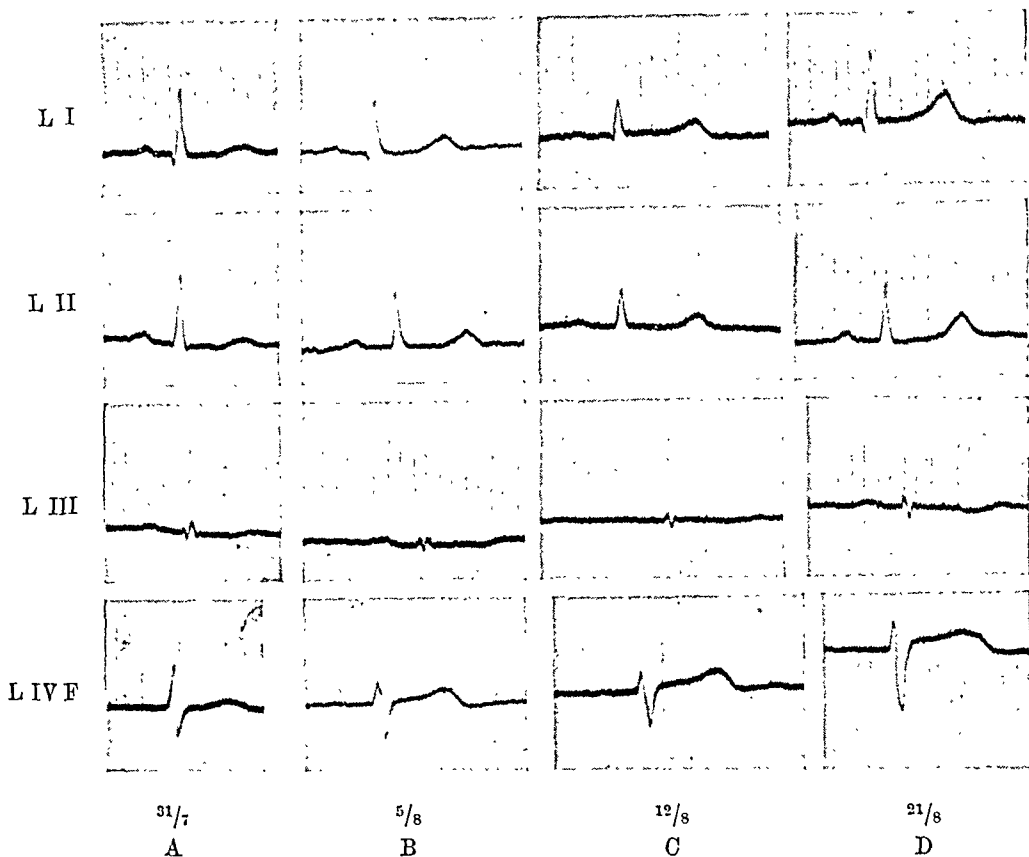


Figure II. Slowly progressing normalisation of a borderline-tracing in pellagra.

On admission (5/6/39), the patient was moderately emaciated; he had 4—5 liquid slightly acid stools; no pathogenic bacterias or parasites were found.

Moderate glossitis marginalis with erosions and atrophy of the papillae of the tongue were present; on the front of the neck a very slight erythema of the skin was scarcely perceptible. There were no other pathological signs, especially with reference to the heart. Psychically the behaviour of the patient was as stated in his past history.

He was treated with Takadiastase, nicotinic acid, 0.20 g by injection and 0.50 g by mouth, and received from 13/6—23/6 10 mg Thiamin per day by injection as well as 2 ccm of a liver and stomach extract.

During this treatment the stools (2—3 per day) became half-formed or formed. The body-weight increased from 33.9 kg to 38.1 kg after an initial loss of 2.4 kg through disappearance of latent edema. There was also an improvement of his psychical behaviour. However, he remained quarrelsome and left the hospital against medical advice.

Comment.

Summary of the electrocardiographic findings.

As shown by the present study, the rapid normalisation of the cardiogram in niacin-treated pellagra according to the pattern described by Rachmilevitz & Braun (19, 20) is often observed, but a number of other developments can be encountered not less frequently.

It results:

1) "Pellagra-cardiograms" can be divided into two groups (Table II):

a) definitely abnormal tracings, which by exclusion of other possible causes and/or the effect of specific treatment can be proved to be brought about by pellagra;

b) borderline-tracings, becoming normal during specific treatment.

2) Pellagra-cardiograms are a frequent effect of the disease. There is no more regularity in the in association of cardiographic alterations with other pellagra-manifestations as in the combination of these manifestations (Table I).

3) The development of the cardiogram during the treatment with nicotinic acid (with or without thiamin, yeast or liver extract), shows various patterns (Tables II and V):

a) complete normalisation,

b) partial normalisation,

c) unchanged persistence of the abnormalities.

4) In most cases a parallelism exists between the general course of the disease and the development of the tracings; but there are many exceptions, the cardiogram may even become worse during clinical cure and improve in fatal pellagra (Tables V and VI).

5) In some instances cardiograms partially normalised during nicotinic-acid treatment were further improved by additional treatment with thiamin or yeast (Table V).

6) The time required for maximal improvement of the tracing during treatment with 0.50 gm nicotinic acid per day varies greatly (Table VII): In half the cases maximal normalisation is obtained within 10 days, in others only after 3—4 weeks.

Factors producing the electrocardiographic changes.

As stressed above, Pellagra is a combined deficiency of B-group factors with predominant symptoms of nicotinic-acid deficiency (and in most cases associated with protein-depletion).

With reference to these facts Weiss & Wilkins (29) assumed, that in Pellagra the cardiographic alterations were entirely due to thiamin deficiency.

Basing on newer observations, Rachmilevitz & Braun (19, 20) attributed the changes to lack of nicotinic-acid.

In our previous paper [Mainzer & Krause (17)], however, we had shown, that thiamin as well as nicotinic acid deficiency, associated in varying proportions, can be effective factors.

The therapeutic experiences related here, tend to confirm this assumption, since in some cases during treatment with nicotinic acid the cardiogram was normalised, whilst in others thiamin (or yeast) was necessary to obtain this result. It remains an open question, how far other B-factors per se or in association are effective, to be resolved only by experiences with isolated factors. Clinical pellagra is not an appropriate object for this analysis.

While thiamin experiments on animals and human beings have been made with the necessary precautions to exclude the development of associated deficiencies [Weiss & Wilkins (29), Weiss, Haynes & Zoll (30), Jolliffe and associates (10)], but with nicotinic acid this has yet to be done.

Pathogenesis of the electrocardiographic changes.

The deficiencies of thiamin and nicotinic acid (both apparently not stored by the organism) produce cardiographic alterations within a very short time; the same is true for their disappearance.

Jolliffe and associates (10) in experiments on human beings found abnormal tracings after four days of induced thiamin deficiency. Swank & Bessey (25) experimenting on pigeons, noted the normalisation of cardiograms even two hours after injection of thiamin. In cardiac Beriberi the influence of thiamin on the cardiogram becomes manifest after a few days, in any case later than the clinical improvement [Weiss & Wilkins (29)]. A similar interval is sometimes sufficient in pellagra during niacin treat-

ment [Mainzer & Krause (17), Rachmilevitz & Braun (20), present observations].

The rapidity of the effect can be attributed to the reversibility of the underlying chemical disturbance. Thiamin and nicotinic acid as moieties of coferments are components of ferment-systems, which together with flavin and adenylyl-tri-(di-) phosphate are necessary for the metabolism of carbohydrates; the degradation of higher carbohydrates provides the energy for the muscular contraction. In this respect the pellagra-cardiogram can be considered as the expression of an abnormal chemism of the myocardial contraction.

Slowly progressing, incomplete and lacking normalisation of the electrocardiogram.

It remains however to discuss, why in many pellagrins the normalisation of the cardiogram progresses slowly, remains incomplete or is even entirely absent, in spite of treatment with the necessary vitamins (or coferments) in excess.

It can be assumed, that in these cases the functional disturbances expressed by the cardiogram are produced by anatomical myocardial damage, more or less reversible at the beginning and irreversible at the end.

A considerable number of investigations support this assumption. Aalsmeer & Wenckebach (1) and Wenckebach (31) reported anatomical and histological heart damage in Beriberi; similar findings were noted by Weiss & Wilkins (29) in pellagra and cardiac insufficiency associated with pellagra or polyneuritis; they found hydropic degeneration of the fibers and interstitial edema of the myocard as described by Aalsmeer & Wenckebach in Beriberi and even in 11 cases of pellagra without cardiac symptoms.

Follis, Miller & Wintrobe (5) reported on myocardial necroses in the thiamin deficiency of the pig, Swank & Bessey (25) in pigeons. Supple, Bender & Kahlenberg (24) found in rats on isolated panthotenic acid deficiency, heart dilatation and myocardial haemorrhage in frequent instances.

Some chronical inflammatory states of the human heart muscle are hypothetically related to nutritional deficiency [Lindberg (13), Smith & Furth (21), Toreson (28)].

So, in spite of inconsistencies and unsettled questions, the occurrence of anatomical myocardial damage in vitamin B-group

Table V.

Development of the electrocardiograms of treated pellagrins.

Nicotinic Acid					Nicotinic Acid + Thiamin					Nicotinic Acid + Yeast					Yeast					Yeast + Liver Extract					No specific treatment					Total
Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
5	1	3	—	15	—	1	—	—	1	2	2	2	—	6	2	1	—	1	4	—	—	—	2	2	—	—	—	1	1	29
																														Inconclusive
																														3
																														Total
																														32

With reference to the presence of coronary diseases, the pellagrins are recorded in groups according to age and sex in table III.

Table IV is a summary of the age and sex distribution of pellagra-cardiograms; 10 series of tracings included in table II are omitted, seven normal ones and three others influenced by coronary insufficiency. The table shows, that in the group of younger pellagrins, abnormal tracings are not less frequent than in the group above 50 years. Hence the statistical analysis confirms the fact derived from the individual examination of every pellagrin, that coronary disease played no major rôle in shaping the abnormal cardiograms.

Table V summarizes the development of the pellagra-cardiogram in treated pellagrins.

Table V refers to the cases of columns 3, 4, 5 and 6a of Table II. However, three of these patients are omitted, owing to incomplete data. From the remaining 24 patients the cardiographic development in 29 courses of treatment is registered. Some pellagrins are registered more than once, if treated at various times by the same or by different procedures, for instance with niacin only at one time, with niacin and yeast in a subsequent period.

Table V shows:

During treatment with nicotinic acid the cardiograms became normal only in a certain number of pellagrins, even if the other pellagrous phenomena disappeared.

In the other cases the normalisation of the tracings remained incomplete in spite of prolonged treatment.

of pigeons. With rapid depletion, induced by complete withdrawal of thiamin, they found no cardiac involvement at the time of natural death; with slow depletion, however, produced by an insufficient amount of thiamin, cardiographic alterations and myocardial damage developed.

Summary.

The electrocardiogram was investigated in 45 pellagrins with 139 tracings.

It was shown:

- 1) There are two groups of pellagra-cardiograms:
 - a) definitely abnormal tracings, which by exclusion of other possible causes can be proved to be brought about by pellagra,
 - b) borderline-tracings, becoming more typical during specific treatment.
- 2) Pellagra-cardiograms are a most frequent effect of pellagra; their association with other pellagra symptoms is irregular.
- 3) There are various patterns of the cardiographic development during nicotinic acid treatment (with or without thiamin, yeast, and liver extract):
 - a) complete normalisation,
 - b) partial normalisation,
 - c) persistence of the abnormalities.
- 4) In most cases a parallelism exists between the general course of the disease and the shaping of the pellagra cardiogram; but there are many exceptions, even the deterioration of the tracing during clinical cure or the improvement of the cardiogram in fatal pellagra.
- 5) Some cardiograms only partially normalised during nicotinic acid treatment, were further improved by thiamin or yeast.
- 6) The time required for maximal improvement of the tracing during intensive nicotinic acid treatment varies from some days to some weeks.

In some pellagrins cardiographic changes are due to thiamin deficiency; in the majority of cases they are probably induced by nicotinic acid deficiency. The influence of nicotinic acid on the cardiogram requires confirmation by experimental work; the same is true with reference to the possible interference of other nutritional factors (B-group, protein-deficiency).

The cardiographic alterations, if quickly reversible, are probably due to an impaired carbohydrate metabolism of the heart-muscle; in cases with delayed or absent reversibility the changes must be related to anatomical myocardial damage.

(The author wishes to express his indebtedness and appreciation to Dr. M. Krause for taking the majority of the tracings.)

References:

- 1) Aalsmeer, W. Ch. & Wenckebach, K. F. (1929), *Wien. Arch. Inn. Med.* 16, 193. — 2) Ellis, L. B. (1946), *Brit. Heart J.* 8, 53. — 3) Feil, H. (1936), *Amer. Heart J.* 11, 173. — 4) Follis, R. H. (1942), *Bull. John Hopkins Hosp.* 71, 235. — 5) Follis, R. H., Miller, M. H. & Wintrobe, M. M. (1943), *Amer. J. Path.* 19, 43. — 6) Follis, R. H., Orent-Keilles, E. & McCollum, E. V. (1939), *Amer. J. Path.* 18, 29. — 7) Gillman, Th. & Gillman, J. (1945), *Arch. Int. Med.* 76, 63. — 8) Graybiel, A., McFarland, R. A., Gates, O. C. & Webster, F. A. (1944), *Amer. Heart J.* 27, 524. — 9) Harris, S. & Harris, S. jr., *Clinical Pellagra*. St. Louis 1941 (Mosby), p. 357. — 10) Jolliffe, N. Goodhart, R., Gennis, J. & Kline, F. R. (1939), *Amer. J. Med. Sc.* 198, 198. — 11) Keefer, C. S. (1930), *Arch. Int. Med.* 45, 1. — 12) Killian, S. T. & Ingelfinger, F. I. (1944), *Arch. Int. Med.* 73, 466. — 13) Lindberg, K. (1938), *Act. Med. Scand.* 95, 281. — 14) McHenry, E. W. & Patterson, J. M. (1944), *Physiol. Rev.* 24, 128. — 15) Mainzer, F. (1939), *Act. Med. Scand.* 100, 208. — 16) Mainzer, F. (1940), *Act. Med. Scand.* 104, 321. — 17) Mainzer, F. & Krause, M. (1940), *Brit. Heart J.*, 2, 85. — 18) Porter, B. & Higginbotham, U. (1937), *South Med. J.* 30, 1. — 19) Rachmilevitz, M. & Braun, K. (1944), *Amer. Heart J.* 27, 203. — 20) Rachmilevitz, M. & Braun, K. (1945), *Brit. Heart J.* 7, 72. — 21) Smith, J. J. & Furth, J. (1943), *Arch. Int. Med.* 71, 602. — 22) Spies, D. T. (1946), *J. Am. Med. Assoc.* 130, 474. — 23) Stewart, C. V. & Manning, S. W. (1944), *Amer. Heart J.* 27, 502. — 24) Supplee, G. E., Bender, C. E. & Kahlenberg, O. J. (1942), *Endocrinol.* 30, 355. — 25) Swank, R. L. & Bessey, O. A. (1942), *Arch. Int. Med.* 70, 763. — 26) Sydenstricker, V. P. (1941), *Ann. Int. Med.* 14, 1499. — 27) Thomas, R. M., Mylon, E. & Winternitz, C. (1940), *Yale J. Biol. Med.* 12, 345. — 28) Toreson, W. E. (1944), *Arch. Int. Med.* 73, 374. — 29) Weiss, S. & Wilkins, R. M. (1937) *Ann. Int. Med.* 11, 104. — 30) Weiss, S., Haynes, F. W. & Zoll, P. M. (1938), *Amer. Heart J.* 15, 206. — 31) Wenckebach, K. F. (1934), *Das Beriberi-Herz*, Berlin (Springer).

The Clinical Value of the Antistreptolysin Reaction.

An Account of the Results of the Reaction in 495
Patients.

By

RICH. AMLIE and PER OEDING.

(Submitted for publication October 17, 1947.)

Methods: The antistreptolysin reaction is carried out by us after the method described by Kalbak in his monography on the same subject. We have, however, used a physiological saline solution for the dilutions, instead of the buffer solution proposed by Kalbak. Dilutions of the serum higher than 1/1,600 have not been made, so that a titre of 1,600 means a maximum titre.

Material: The reaction was introduced as a routine examination in our laboratory during the summer 1944, but the number of titrations was at first very small. As the knowledge of the reaction steadily been increasing, the number of titrations has increased among the physicians, so that we now examine about 30 serums weekly.

The material consists of 670 titrations in 495 patients. In most of the cases thus only one titration in each patient has been made, a drawback which we will return to later on. The blood-samples have been sent us from hospitals from different parts of Norway, but the great majority have come from hospitals in Oslo.

We have thus had the opportunity of examining most of the case histories ourselves. To the clinics away from Oslo we sent a questionnaire, where we listed the data desired about the patient. Special stress was laid on getting the diagnoses at discharge from hospital, and not only the diagnosis on admission. Further

we got information of the age, the duration of the disease, prodromes, phase (active-inactive, acute-chronic), probable etiology, the condition of the patient and special findings (S. R., blood pressure, urine analysis etc.) at the date when the titration was made. Only of relatively few patients have we got incomplete information.

Most of the previous investigations are based on selected materials of well defined clinical conditions, as for instance the investigations of Kalbak. It is evident that such a selected material will give much more favourable statistics than an unselected material, as the number of wrong diagnoses is reduced to a minimum, and atypical cases are not included in the material. A weakness of this method is, however, that the investigator often knows the diagnosis of the patient, whose serum he titrates. The reading of the titre may thus be unconsciously influenced, as it may be a matter of opinion whether one chooses the titre of 180 or 200, and the higher the titre, the greater the possibility of error.

Another circumstance is that previous investigations in a less degree have met the requirements of the physician. To him the reaction will be of particular interest in the clinically obscure, atypical cases, not in the certain ones, where the diagnosis already is clear.

In addition to the knowledge which earlier reports of the antistreptolysin reaction in a selected material have accumulated, we have felt that it would be of the greatest interest to see the results of the reaction in an unselected material, such as it appears in a routine laboratory.

However, our material also has resulted from selection in so far as the serums mostly were taken from patients suffering from conditions, in which the physician had reason to expect an elevated titre. That applies especially to obscure diseases of the joints and kidneys, »rheumatic» conditions and obscure fevers.

In order to facilitate comparison of our results with those of previous authors, we have mainly used the same classification as these. A great part of our cases has been difficult to classify, either because the diagnosis was obscure or due to simultaneous occurrence of a number of different diseases. We have therefore divided into separate groups acute and chronic cases of nephritis and rheumatic fever, and some other diseases of special interest, while the rest of the cases have been collected in a mixed group.

The literature contains somewhat different views concerning the highest normal limit of the A. S. T. We have, like Kalbak, considered an A. S. T. above 200 as increased. It must, however, be clear that a titre of 150—180 may be a normal one, or it may be an increasing or decreasing titre. In order to decide this the results of more samples from the patient must be compared.

I. Diseases of the joints.

1. *Acute polyarthritis (rheumatic fever).*

This group consists of altogether 79 patients, of these 32 were men and 47 women. In 20 of them information of previous attacks was given. In 36 patients, viz. 46 %, information of a pre-disease was given, in most of the cases a sore throat. Only 16 could state fairly accurately the interval between the predisease and the outbreak of the rheumatic fever. The average interval in these was 17 days.

A. S. T.....	0—99	100—199	> 200
Number of patients.....	3	6	70

In 89 % of the patients the A. S. T. is increased. It might be of interest to examine the clinical data in the 9 patients with normal titres (11 %), to see if they, also in other respects, differed from the others.

Pat. nr. 8: Age 60, woman. 29th week of disease: A. S. T. 180.

Pat. nr. 14: Age 26, woman. »Very light case». 1st week of disease: A. S. T. 100, S. R. 10.

Pat. nr. 20: Age 49, man. Normal course of disease. 5th week of disease: A. S. T. 190, S. R. 64.

Pat. nr. 37: Age 40, man. 2nd week of disease: afebrile, free from symptoms, A. S. T. 50, S. R. 4.

Pat. nr. 49: Age 37, man. Myalgia of short duration, rheumatismus acutus? A. S. T. 180, S. R. 10.

Pat. nr. 60: Age 45, man. »Light degree». 2nd week of disease: A. S. T. 50, S. R. 43.

Pat. nr. 65: Age 49, woman. 17th week of disease: A. S. T. 100, S. R. 20.

Pat. nr. 69: Age 22, woman. »Light degree». 1st week of disease: A. S. T. 170, S. R. 10.

Pat. nr. 70: Age 49, woman. 6th week of disease: A. S. T. 90, S. R. 6.

It is evident that most of these cases are atypical in one or other respect. Already the fact that as many as 6 are more than 40 years old is striking. 3 were characterized as clinically mild cases, of whom 2 had an S. R. of 10 when the A. S. T. was made. Further 2 were symptom free with a normal S. R. and in 2 cases the diagnosis was obscure. The remaining 2 patients, nr. 8 and nr. 20, both apparently had a normal course of disease, but with A. S. T. on the upper limit. In all these cases only one titration was made, and it is possible that more titrations in the same patient at different times might have given other results. Thus, in 2 more patients with titres over 200, a previous analysis had shown normal values.

The A. S. T. will ordinarily be increased already during the first week. The increased titre is, however, an answer to the pre-disease, mostly a sore throat, so that the duration of the disease is, in fact, 2—3 weeks longer. The titre will ordinarily not reach its upper limit until 2—3 weeks after the beginning of the joint symptoms, and frequently much later. The A. S. T. is increasing and falling more slowly than the S. R. (see Fig. 1) and may be increased for a long time after the S. R. and temperature have become normal and the patient free from symptoms. This is, probably, due to a remaining focal infection, which is continuing to produce antibodies after the patient, to all appearances, has been cured, and which represents a danger of relapse. Kalbak calls attention to this fact and is of the opinion that the A. S. T. ought to be controlled after the clinical recovery, until the titre is normal or shows a distinctly falling tendency.

It has been discussed if there exists any connection between the clinical course of the rheumatic fever and the A. S. T. While some authors have thought that they could demonstrate such a connection, others, among them Kalbak, could not find higher titres in the serious cases than in the mild. Kalbak divided his cases in mild, medium and serious, a classification which necessarily had to be rather arbitrary.

We have chosen to pick out the particularly mild and the particularly serious cases to see if these clinical extremes show any difference in the titre.

Out of 8 particularly mild cases the A. S. T. was below 200 in 5, moderately increased in 2 and strongly increased in 1.

Out of 6 serious cases 4 were complicated with exudative pleurisy and pericarditis, and 1 of them died. 3 of them showed

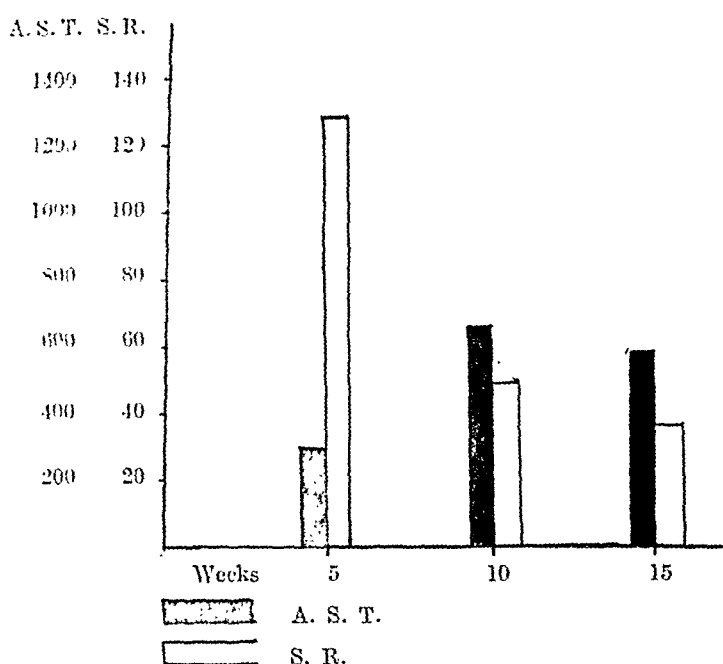


Fig. 1. The relation between A. S. T. and S. R. in patient nr. 67, rheumatic fever.

titres above 1,000, the 4th had an A. S. T. of 400, but as early as in the 3rd week. The 5th case was a grave, progressive one with A. S. T. of 900. The 6th patient will be described more closely:

Pat. nr. 5: Age 15, woman. December 1945 the patient contracted scarlet fever at the same time as her brothers and sisters, while the mother got a panaritium. In the beginning of January 1946 she got rheumatic fever and in the last days of January cardiac symptoms. A. S. T.: 10/8 46 = 1,600, 15/8 46 = 1,600. Died 20/8 46. From the heart valves hemolytic streptococci, group A, type 19 could be isolated.

It can thus be no doubt that there exists a correlation between the clinical course and the A. S. T. in our material, as the light cases generally give low titres and the gravest high titres. We have, however, not been able to demonstrate a higher A. S. T. in the relapses than in the first attack.

It might be of interest to mention 2 patients where tonsilectomy was performed during the stay:

Pat. nr. 1: Age 33, woman, no previous attack of rheumatic

fever. 30/11 45: duration 17 weeks, A. S. T. 100, S. R. 37. 5/12 45: tonsilectomy. 15/12 45: A. S. T. 250, S. R. 18.

Pat. nr. 76: Age 44, woman, rheumatic fevers several times during 15 years. Last 3—4 years frequently sore throat. After angina, acute relapse 18/7 44. 25/7 44: A. S. T. 1,000, S. R. 34. 10/8 44: tonsilectomy. 14/8 44: A. S. T. 1,600, S. R. 45. 23/8 44: A. S. T. 225, S. R. 39.

Both patients get an increase of A. S. T. after the tonsilectomy. Patient nr. 76 in the next sample, 9 days later, shows a great fall of A. S. T. It seems as if the tonsilectomy temporarily has activated the focus.

In 2 patients the A. S. T. was examined in exudate from the knee joint. It was a good accordance between the A. S. T. in blood serum and exudate from the knee joint.

Antibodies could, however, not be demonstrated in the spinal fluids (A. S. T. = 0) in 4 patients, while the titres in serum were elevated, and in one case reached 600.

2. Polyarthrititis chronica.

The group consists of 80 patients, of these 31 men and 49 women.

Table 1.

A. S. T. in patients suffering from chronic polyarthrititis.

	A. S. T.			
	0—99	100—199	>200	Sum
Primary	3	7	0	10
Secondary	6	7	39	52
Obscure	5	5	8	18
Total	14	19	47	80

The difference of A. S. T. in primary and secondary polyarthrititis is striking and will be seen in table 1. All 10 patients with primary, chronic polyarthrititis have normal A. S. T. Among 52 patients with secondary, chronic, rheumatic polyarthrititis 39 have an A. S. T. above 200, while in 13 patients the titres were not increased.

It may be of interest to see how A. S. T. is related to the active and inactive cases, *i. e.* if the cases of chronic, secondary polyarthrititis are connected with an increased A. S. T., and the inactive

cases with a normal A. S. T. The cases are, furthermore, classified in early and late ones. As the patients have come to the hospital on account of their joint complaints it is evident that the greater part of the patients are in an active phase. Some are in an inactive phase, either because they have come to hospital on account of another disease, or because the disease has become stationary during the stay.

The classification of some of the cases may be rather arbitrary, and sometimes there was some doubt whether the phase should be called active or inactive. The classification has been made on the basis of the clinical state and the S. R., regardless of the A. S. T. 25 cases of questionable etiology and classification were excluded.

Table 2.

A. S. T. in different phases of chronic polyarthritis.

		A. S. T.			
		0-99	100-199	>200	Sum
Polyarthritis	early case.....	1	3	0	4
chron. prim.	late case {active	1	3	0	4
	{inactive ..	1	0	0	1
Polyarthritis	early case.....	0	0	1	1
chron. sec.	late case {active	3	6	31	40
	{inactive...	2	1	2	5
Total					55

In a total of 55 cases, among them 9 cases of primary chronic polyarthritis and 46 cases of secondary chronic polyarthritis, this classification was done.

All cases of primary chronic polyarthritis showed a normal A. S. T., both in early and in late, active and inactive phases.

Regarding the cases of secondary, rheumatic polyarthritis a marked difference of A. S. T. in the active and inactive phases was noticed. Among 40 cases in an active phase 31 showed an increased A. S. T., and among 5 cases in an inactive phase 2 were increased.

3. *Other diseases in the joints and muscles.*

In this group are included all other cases of diseases in the joints and elsewhere in the organs of locomotion. We must as-

sume that a great part of the cases are atypical and the diagnoses correspondingly unclear, since it has been found desirable to determine the A. S. T. The material has been classified in sub-groups, to study the results of the A. S. T. in diseases where hemolytic streptococci are not known to play any etiological rôle. These sub-groups can, however, not be used as a normal control material, mostly because of the possibility of erroneous diagnoses.

Table 3.

A. S. T. in various diseases of the joints and muscles.

	A. S. T.			
	0-99	100-199	>200	Sum
Bechterew's disease	3	3	6	12
Reiter's disease	1	2	6	9
Still's disease	1	0	2	3
Rheumatismus ac. atyp.	0	1	13	14
Peritendinitis	0	1	1	2
Periarthritis	1	1	0	2
Non rheumatic arthritis	0	4	4	8
Ischias	3	1	1	5
Lumbago	1	2	1	4
Arthrosis, spondylosis	6	8	4	18
Osteoporosis	1	2	0	3
Bone tumours	0	1	1	2
Other diseases	2	10	22	34
Total	19	36	61	116

Among 12 patients suffering from *Bechterew's disease* 7 were men and 5 women. 3 of them had previously suffered from rheumatic fever. In 8 of the patients more than one titration has been made. 6 of the patients had an increased A. S. T. It is a question if special conditions, which might explain the increased A. S. T., can be found in these cases.

The duration of the disease does not seem to be of any significance, as 2 of the 3 patients in whom the duration was less than 1 year had normal titres. Nor does there seem to be any relation between the S. R. and the A. S. T.

In the 6 patients who have an A. S. T. above 200, this is to be noted:

Pat. nr. 163: Age 21, man. Simultaneously suffering from a chronic polyarthritis.

Pat. nr. 166: Age 16, man. 3 years ago rheumatic fever. At

the time when the A. S. T. was made hemolytic streptococci were isolated from his throat.

Pat. nr. 167: Age 44, woman. The diagnosis Mb. Bechterew doubtful.

In 3 of the 6 patients with an increased A. S. T. this may thus be due to other conditions. In the remaining 3 cases nothing of special interest was found.

Out of 9 patients suffering from *Reiter's disease* 6 had an increased A. S. T. This result is in accordance with the findings in rheumatic fever and chronic, secondary polyarthrititis, and suggests that hemolytic streptococci may be of etiological significance in Reiter's disease. No relation between the S. R. and the A. S. T. was demonstrated. In all 3 cases where A. S. T. was not increased the S. R. was more than 50 mm.

Still's disease: In 2 of the patients, who were in an active phase of the disease, A. S. T. is increased. In one of them hemolytic streptococci were demonstrated in the throat. In the third case the A. S. T. was 90 (*Pat. nr. 182*). This was a 3½ years old girl, who had suffered from rheumatic fever about 10 months earlier. She was now in a clinically inactive phase of the disease, but with an S. R. of 34 mm.

In these 3 cases of Still's disease the A. S. T. thus behaves as in chronic, secondary polyarthrititis.

Peritendinitis: 1 case with an increased A. S. T. followed as a complication to an acute maxillary sinusitis, and disappeared with the treatment of the sinusitis.

Arthrosis, spondylosis: 4 patients showed an increased A. S. T. Among these 1 in addition had an infiltration of the lung, and in 2 the A. S. T. showed border-line values (200, 240).

Bone tumours: In 1 patient with cancer metastases to the columna A. S. T. in the first sample was 800, in the next only 40.

Other diseases: This sub-group includes several different conditions, partly of obscure etiology. Many of them are inflammations (osteomyelitis, spondylitis etc.), where an increased A. S. T. can be expected.

II. Urinary complaints.

1. Acute nephritis.

21 patients suffering from acute nephritis were examined. In 8 of them information of preceding disease was given, viz.: angina

tonsillaris 2, pyodermia 2, infected wound 1, mastitis 1, osteomyelitis 1, scarlatina 1.

A. S. T. was increased in 18 of the patients, all typical cases. Among the 3 patients where A. S. T. was not increased, 2 were typical cases, while the 3rd was characterized as atypical, with few objective findings.

In the cases where more titrations were done in the same patient, we could find no parallelism between A. S. T. and blood pressure-urine findings. A. S. T. is already increased in the first days of the disease and may remain increased for months, even a long time after the blood pressure and the urine have become normal.

In such a small material it is difficult to find out if the serious cases are accompanied by a high A. S. T. and the light cases by a low titre. The only one of our cases which was characterized as light, however, had a normal A. S. T., while the only one of the patients who died, had a titre as high as 1,000. This was in the 5th week of the disease, 3 days before death.

2. Chronic nephritis.

Among 7 patients with chronic nephritis 6 were in an active phase and 1 in an inactive phase, the last one with an A. S. T. of 40. Among the 6 active cases 4 had a moderately increased A. S. T., the highest titre was 280, viz. much lower than in acute nephritis. In 2 patients the A. S. T. was not increased. One of them was a patient with subchronic nephritis in the third month, after pregnancy, and the other a case of strongly nephrotic character.

Table 4.

A. S. T. in diseases of the urinary tract.

	A. S. T.			All
	0-99	100-199	>200	
Acute nephritis	2	1	18	21
Chronic nephritis	1	2	4	7
Other urinary complaints	11	6	7	24

3. Other urinary complaints.

Among 24 patients suffering from other diseases in the urinary tract (nephrosis, cystitis, pyelitis, albuminuria, hematuria) 17 had a normal and 7 an increased A. S. T. Of these 7, 4 are only

doubtfully increased, with titres of 200, while 3 are distinctly increased. These are:

Pat. nr. 313: A. S. T. 480. 2 years ago acute nephritis, now pains in the back, albuminuria.

Pat. nr. 316: A. S. T. 350. Hematuria of unknown etiology, reduced clearance.

Pat. nr. 322: A. S. T. 1,000 and 1,280, pyelitis.

In all these 3 cases hemolytic streptococci may have played a rôle. The group contains 3 cases of nephrosis, all with a normal A. S. T.

Table 4 distinctly shows the difference of A. S. T. in the 3 groups of urinary diseases, in accordance with the results of earlier authors. In acute nephritis the A. S. T. is nearly always increased. In typical cases where A. S. T. is not increased it has been assumed that other microbes than hemolytic streptococci are the cause of the disease. In chronic nephritis the frequency of increased A. S. T. is smaller, while only a small part of patients with other urinary complaints have an increased A. S. T.

III. Angina tonsillaris.

A. S. T.	0-99	100-199	>200
Number of patients	0	0	6

In accordance with earlier investigations all 6 patients suffering from angina tonsillaris show an increased A. S. T. In 2 of the patients the A. S. T. was increased already in the first week (600, 220).

IV. Erythema nodosum.

Table 5.

A. S. T. in erythema nodosum.

	A. S. T.		
	0-99	100-199	>200
Tuberculous etiology	1	3	2
Rheumatic etiology	0	0	3
Obscure etiology	0	0	3

A. S. T. has been determined in 12 patients suffering from erythema nodosum, 1 man and 11 women. In some of the cases the etiology of the disease may have come into a different light after the examination of A. S. T. We have classified the material according to the clinical diagnosis, regardless of the A. S. T.

Among 6 cases of a probably tuberculous origin 4 have a normal A. S. T. and 2 an increased A. S. T., 1 of which recently had a sore throat. All 3 cases of erythema nodosum of a probably rheumatic origin show an increased A. S. T., and 3 cases with an obscure etiology are also increased. Even if the material is small it definitely shows that A. S. T. is increased in erythema nodosum of rheumatic origin, and for the most part normal when the origin is tuberculous. But as the tuberculous primary infection frequently is accompanied by infections in the nose and throat, such as an angina tonsillaris, which may increase the A. S. T., this circumstance must be taken into consideration when the result of the titration is judged. The value of the reaction is thus a little doubtful in erythema nodosum.

V. Erythema multiforme.

A. S. T. was examined in 4 women suffering from erythema multiforme.

A. S. T.	0-99	100-199	>200
Number of patients	1	1	2

In 2 patients A. S. T. was increased. The first one had suffered from a sore throat for a long time, and the second patient had recurrent pains in the joints for 1 year, with an acute relapse. In these 2 cases a rheumatic origin is thus quite probable from a clinical point of view, and the results of A. S. T. support it.

VI. Pleurisy.

A. S. T.	0-99	100-199	>200
Number of patients	1	0	3

Among 4 patients suffering from pleurisy of obscure etiology, 3 had an increased and 1 a normal A. S. T.

VII. Other diseases.

The group consists of 133 patients with different diagnoses, which have not been mentioned above. As far as possible we have classified the cases in sub-groups.

Infections, where hemolytic streptococci were isolated. In 2 patients the A. S. T. was increased to 600. The first case was a septicemia

where hemolytic streptococci were cultivated from the spleen post mortem. The second case was a pericarditis where the microbe was cultivated from the pus. In the third case the A. S. T. was 180 during the second week. This was a boil where hemolytic streptococci were isolated from the pus.

Table 6.
A. S. T. in a mixed group of diseases.

	A. S. T.			
	0-99	100-199	>200	Sum
Infections, hem. streptoc. +	0	1	2	3
Other acute infections	2	4	12	18
Scarlatina	0	0	1	1
Septicemia, pyemia	0	0	4	4
Sinusitis	1	0	3	4
Febris catarrhalis	0	1	4	5
Febris causa ignota	3	6	3	12
Pneumonia	2	2	9	13
Purpura rheumatica	1	0	0	1
Mononucleosis infectiosa	0	1	0	1
Asthma bronchiale	1	1	1	3
Meningitis chronica	0	0	6	6
Boeck's sarcoid	0	0	3	3
Lymphogranulomatosis	0	0	1	1
Myelomatosis	0	2	0	2
Leukemia	1	1	0	2
Cancer, sarkoma	1	1	4	6
Other diseases	14	15	19	48
Total	26	35	72	113

1 case of *scarlet fever* showed an increased A. S. T., which was to be expected.

4 cases of *septicemia*, in which no microbes had been isolated, all showed an increased A. S. T.

Among 4 cases of *sinusitis* 3 showed an increased A. S. T. (200, 250, 480). That can easily be explained from the fact that hemolytic streptococci are frequently cultivated from patients suffering from sinusitis.

Febris catarrhalis: 5 cases, among them 4 increased (140, 200, 200, 320, 400).

Among 12 cases of *febris causa ignota*, however, only 3 are increased.

Among 13 cases of *pneumonia* as many as 9 are increased, with titres between 200 and 1,280. 6 cases are complicated (poly-

arthritis 2, pleurisy and pericarditis 4), 5 of which show an increased A. S. T.

It is extraordinary that all 6 cases with the diagnosis »chronic meningitis» have a definitely increased A. S. T., between 300 and 1,600.

Boeck's sarcoid: 3 cases, all with an increased A. S. T. (400, 220, 850). In one of the patients the diagnosis is: »lymphadenitis, Boeck's sarcoid?»

In the sub-group *cancer, sarkoma* 4 of the 6 patients have an increased A. S. T. That can probably be explained as an increased frequency of streptococcal infections, due to the lowered general condition of the patients.

Summary and Conclusions.

1. The antistreptolysin reaction has been done in 495 patients. The material is mixed and unselected, and comprises all titrations made in the institute. Previous authors most frequently have examined a selected material. We were of the opinion that it might be of interest, especially to the physician, who makes use of the reaction just in the atypical cases, to investigate an unselected routine material.

2. The reaction was performed after the technique described by Kalbak. An A. S. T. above 199 was regarded as increased.

3. A. S. T. was increased in 89 % of the patients (79) with rheumatic fever. A definite correlation between the course of the disease and the A. S. T. was found, with low titres in the mild and high titres in the severe cases.

4. Among 80 patients suffering from chronic polyarthritis all 10 patients with primary, chronic polyarthritis had a normal A. S. T. In 52 patients with secondary, chronic polyarthritis A. S. T. was increased in 39 cases, and by preference in the active cases.

5. 116 patients, suffering from other diseases in the joints and muscles, were examined. A. S. T. was increased in 61 cases. In most of the cases where A. S. T. was increased this was in agreement with the etiology and course of the disease.

6. Among 21 cases of acute nephritis the A. S. T. was increased in 18.

7. 4 out of 7 patients with chronic nephritis showed a moderately increased titre.

8. Among 24 patients suffering from other diseases in the urinary tract 7 showed an increased A. S. T., whereof 4 were doubtful, with titres of 200 only.

9. 6 patients with angina tonsillaris all had an increased A. S. T.

10. In 12 patients suffering from erythema nodosum the A. S. T. was examined. When the etiology was rheumatic the titre was increased, and in the tuberculous cases mostly normal. A. S. T. may be increased in tuberculous erythema nodosum too, due to infections in the nose and throat which frequently accompany the primary infection.

11. Among 4 cases of erythema multiforme A. S. T. was increased in 2, in which, also clinically, a rheumatic origin was probable.

12. The A. S. T. was increased in 2 of the 3 cases of pleurisy, where the etiology was obscure.

13. Among 133 patients suffering from other diseases the A. S. T. was increased in 72 cases, most of which were infections where hemolytic streptococci could be expected to have played a rôle.

14. The reaction is a valuable aid to the physician, especially in obscure diseases of the joints and kidneys. More than one sample from each patient must always be examined, and the results must not be judged alone, but in relation to the clinical state.

References.

Kalbak, K.: Undersøgelser over O-Streptolysin og Forekomsten af O-Antistreptolysin i serum. Ejnar Munksgaards Forlag, Copenhagen, 1942. — Further references are found in Kalbak's book.

Publications Received.

Redaktionen sänder på anmodan böcker för recension.

Bulletin of the Institute for Medical Research of the University of Madrid. Vol. 1 num. 1—2, 1948.

The Tohoku Journal of Experimental Medicine. Vol. 49. Nos. 1—2. 1947. The Tohoku University, Sendai, Japan.

Archivos Cubanos de Cancerologia, Organo oficial del Instituto del Radium. Vol. 6, Nos. 10—11—12, 1947, Vedado, Habana, Cuba.

Joseph J. McDonald, Joseph G. Chusid and Jack Lange: Correlative Neuroanatomy. 4th edition, revised. 156 p. 60 ill. Price: \$ 3.00. University Medical Publishers, Palo Alto, California, 1948.

V. J. Kinsella: The mechanism of abdominal pain. 230 p. 17 fig. Price: 32/6. Australasian Medical Publishing Co. Ltd., Sydney. 1948.

V. J. Kinsella: »Elective alimentary rest» and The elimination of so-called »Paralytic ileus» after abdominal operations. 35 p. Price: 3/—. Australasian Medical Publishing Co. Ltd., Sidney. 1948.

Sven E. Donner: Intracranial acute edema. 79 p. Acta psychiatrica et neurologica. Suppl. 51. Ejnar Munksgaard. Copenhagen 1947.,

Claudio Cervini: Alimentazione e tumori. 111 p. Rivista »Omnia medica». Vol. XXVI. N. 1—3, 1948.

Kaare Lilleengen: Typing of Salmonella typhi murium by means of bacteriophage. 125 p. 23 fig. Acta pathologica et microbiologica scandinavica. Suppl. LXXVII. Ejnar Munksgaard, Copenhagen, 1948.

Ragna Rask-Nielsen: On the development of tumors in various tissues in mice. 144 p. 14 fig. Acta pathologica et microbiologica scandinavica. Suppl. 78. Ejnar Munksgaard, Copenhagen, 1948.

- Pathologie du pancréas.* Rapports et discussions du 2:me congrès belge de chirurgie et de la séance extraordinaire de la Soc. belge de gastro-entérologie du 12 Juin 1948, à l'Université de Louvain. Les Editions »Acta Medica Belgica«, Bruxelles, 1948.
- De nordiska kriminalistföreningarnas årsbok 1946—47.* Yearbook of the northern criminalists. 383 p. Ivar Haeggströms Boktryckeri A.-B., Stockholm 1948.
- P. van der Meer and J. Zeldenrust:* Reticulosis and reticulosarcomatosis. 83 p. 21 fig. Price: fl. 4.90. Universitaire Pers Leiden, Leiden 1948.
- C. Jimenez Diaz:* Lecciones de patología médica. (Enfermedades del Hígado). Tomo VI. 998 p. 358 fig. Editorial Científico-Médico, Madrid-Barcelona, 1948.
- René Charry:* Chirurgie moderne de la hanche. Apport de la radiographie de profil. 326 p. 512 fig. Prix: 1.340 fr. G. Doin & Cie, Paris, 1948.
- J. Rieux et J. Bouillot:* Traité des maladies professionnelles. 466 p. avec figures. Prix: 1.100 fr. G. Doin & Cie, Paris, 1948.
- Louis Gerson:* Les varices et leurs associations pathologiques. 2:e édition. 260 p. 57 fig. Prix: 850 fr. G. Doin & Cie, Paris. 1948.
- Robert Hegglin:* Die Klinik der energetisch-dynamischen Herzinsuffizienz. 120 S. 82 Abb. Preis: sw. Fr. 19.—. S. Karger, Basel & New York, 1947.
- Lavori dei Congressi di Medicina Interna.* XLVIII Congresso tenuto in Roma 22—25 Ottobre 1947. Ditta Luigi Pozzi, Editore, Roma, 1947.
- Maurice Lamy, Michel Lamotte et S. Samotte-Barrillon:* La Dénutrition. 407 p. Prix: 1.150 fr. G. Doin & Cie, Paris. 1948.
- Umberto Niccolini:* Fisica delle Mesoforme. 24 p. Istituto Radiazioni Elettromagnetiche, Milano, 1948.
- Umberto Niccolini:* Fisica Ottica e Teoria dell'Urto. 24 p. Istituto Radiazioni Elettromagnetiche, Milano, 1948.
- John O. Haman:* Artificial Insemination. California Medicine, Vol. 68, No. 5, 1948.
- Hawaii Medical Journal,* Vol. 7, No. 3—5, 1948. Official publication of the Hawaii territorial medical association.

The Central Clinical Laboratory of Södersjukhuset, Stockholm.
(Greta Hammarsten, M. D., Physician-in-Chief)

Are Non-Nucleated Erythrocytes Formed by Budding off of Cytoplasm from Normoblasts?

By

LISA BOSTRÖM.

(Submitted for publication October 8, 1947.)

Ever since Neumann in 1868 demonstrated nucleated red blood corpuscles in the bone marrow, it has been assumed that the non-nucleated erythrocytes of the blood are formed from these elements. Thus it has been supposed that the blood corpuscles develop from proerythroblasts into acidophil normoblasts which later become denucleated.

The fact that the nucleated cells of the marrow are not sufficient to supply the enormous amount of new red cells required every day — about 25×10^{10} — has been assumed to be compensated for by a rapid proliferation of normoblasts. But what becomes of all the nuclei?

Schilling (1) suggested that the thrombocytes were the cast off nuclei of erythrocytes. Schilling's »Plättchen» theory, however, collapses with Wright's interpretation of thrombopoiesis as bound up with the megakaryocytes. No support for the older conception of karyorrhexis and karyolysis is found in normal marrow. Cellular nuclei down to 3 or 4 μ in diameter are seen, and then they disappear completely (1). On the other hand, free nuclei and eccentrically situated ones are often seen in the marrow. According to Rohr (2), one is forced to assume from these observations that »the denucleation takes place through the extrusion of the nucleus».

As late as 1944 Leitner (3) expressed his agreement with Naegele's (4) theory of intracellular nuclear disintegration. In 1938 Schilling (5) pointed out the obscurity on this point. After mentioning lacunae in other fields of hematology, he said: »What is still more remarkable is that the question of the denucleation of the red cells is still unsettled. Most histologists ignore this point or keep to the old conception of intracellular disintegration, though one never sees the necessary intermediate stages, or of nuclear extrusion, convincing pictures of which are only seen in toxic anemia. Whatever the case, the nucleated erythroblast, which is so difficult to digest, must be denucleated by some organic process of maturation. *Otherwise the whole theory that the non-nucleated red blood corpuscles come from nucleated ones is wrong.*» (The italics are Schilling's.)

The important problem of the way in which the erythrocytes pass from the parenchyma of the bone marrow into the circulating blood is also unsolved. It has been a moot question whether the vascular system in the bone marrow is open or closed. First it was believed that the marrow lacked an orderly vascular system. Injections of india ink, however, have demonstrated the presence of a distinct capillary net. In 1922 Doan (6) proved, in his opinion, that the vascular system was closed, and most histologists since then agree with him. Rohr (2) said that the parenchyma of the bone marrow was »lückenlos geschlossen» in relation to the bone marrow.

The American scientists, Doan, Cunningham and Sabin (7) assumed that the red blood corpuscles are formed intravascularly in vascular areas which were cut off from the circulation. When the blood corpuscles are mature, they reasoned, these reserves open up. They did not present any microscopic support for their assumption, which is naturally based on Sabin's (8) fascinating observation of the formation of blood corpuscles inside the blood vessels.

Maximow (9) said that in all mammals the red blood corpuscles are formed in the reticular tissue between the vessels. Turnbull (10) said that there was no doubt that erythropoiesis in human marrow takes place extravascularly. This is seen clearly, he stated, when the reticulum is stained with silver. The erythropoietic cells are then seen lying in the reticulum outside capillaries, often directly beside them. Groups or islands of these cells are often traversed by fibrils of the reticulum.

deficiency and in clinical pellagra, associated or not with cardiac symptoms, has been definitely proved.

Absence of parallelism between the electrocardiographic alterations and other pellagra manifestations.

In the pathogenesis of the pellagra-tracing, the absence of parallelism between the general trend of the disease and the cardiographic alterations is a further unsettled problem.

The fact has already been noted by Weiss & Wilkins (29). They observed severe heart failure with or without abnormal tracings and in other cases cardiographic alterations without cardiac symptoms; they noted similar irregularities with reference to anatomical myocardial damage. In the induced thiamin deficiency of rats Weiss, Haynes & Zoll (30) found in different experiences with the same animal various patterns of cardiographic deformations or absence of abnormalities.

We encountered the same lack of conformity in pellagrins. Actually we have never seen serious pellagra of longer duration without abnormal tracings; this could be anticipated, since pellagra cardiograms were present in three quarters of the patients; however, there was no relation between the presence or intensity of the cardiographic changes and the duration, severity or predominant localisation of the pellagra manifestations. During treatment there was mostly a parallelism between the electrocardiographic development and the general trend of the disease; however, we have reported several notable exceptions.

We assume, that these irregularities are due to the interaction of other B-factor deficiencies; deficiency of a second factor can foster as well as prevent the production of myocardial damage produced by a primary deficiency; this unexpected fact is shown by experiments of Follis, Orent-Keilles & McCollum (6), of Follis (4) and of Thomas, Mylon & Winternitz (27).

The question, if protein deficiency — a frequent condition in pellagrins — modifies the influence of B-factors on the cardiogram has not been systematically investigated. In a case of extreme hypoproteinemia, however, observed by Killian & Ingelfinger (12) the tracing was normal.

Moreover the speed of the depletion has a bearing on the clinical phenomena of a deficiency disease. This was experimentally shown by Swank and Bessey (25) in the thiamin deficiency

LISA BOSTRÖM.

ward Habelmann (15) observed the detachment of protoplasm from macroblasts and believed this to explain the origin of poikilocytes. These observations were made in stained smears and were therefore criticized by Froboese (16). As he pointed out, it is unwise to draw conclusions about the formation of cells from stained smears, for there the protoplasm may be stretched into any imaginable shape.

The term poikilocytosis was originally meant to cover irregularity in the outline of the blood corpuscles *in vivo*. Since then the term has gradually been extended to embrace a large number of artefacts arising *in vitro* in a more or less non-physiologic environment. This has caused great confusion. It is well known how easily the erythrocytes lose their disk shape. Groups of crenated blood corpuscles around dust grains, air bubbles and so on are often seen in living blood. The nicking of the edge often observed represents the first phase in the transition from the disk to the crenated shape (17). Spherical blood corpuscles should naturally never be considered as poikilocytes.

If all kinds of irregularity in the outline of blood corpuscles are included in the term poikilocytosis, it is no wonder that the condition is denied any diagnostic significance (4). When the artefacts are excluded, however, there remains a genuine poikilocytosis which can be observed in both living and smeared blood. There is every reason to consider this irregularity as a sign of abnormal erythropoiesis.

Only well spread and rapidly dried preparations can be used for evaluating the degree of poikilocytosis. The spreading should be done as slowly as possible on well cleaned, polished slides so as to prevent overlapping of the blood corpuscles. The slides should be fanned immediately after the smear is made so that the blood is quickly dried. The edges of the smear should not be taken into consideration when the preparation is examined.

The poikilocytes which are assumed to develop according to the present working hypothesis are most common in pernicious anemia. In stained smears they are seen as well-shaped megalocytes in the shape of a pear, the tapering end of which has a long or short process. The outlines are clear and smooth, showing no notches or scars (fig. 4 a).

The same type of poikilocyte is seen in a number of other anemias as well as in leukemia and hemolytic icterus. The processes may vary in length and thickness. All types of transitional forms

are seen, from vermiform appendages (fig. 4 b) to the tack-like processes of the normocytes (fig. 4 c). Drop-shaped corpuscles with a blunt, tapering place of attachment instead of a process are also seen (fig. 4 d).

Poikilocytes can be visualized in living blood as well. There the processes are seen projecting gracefully from the edge of the rouleaux. The external tip is generally thickened into a bud. The process is seen as a permanent deformity, remaining the same however the corpuscle whirls about in the blood. The gentle rounding at the base of the stalk is characteristic. Straight outlines are only observed when the elastic corpuscle is stretched after having fastened on some unevenness in the slide.

It is seen clearly in living blood that the poikilocyte is disk-shaped. Thus in reality it is not pear-shaped or drop-shaped as it appears in smears. Instead it has the shape of a racket or hand mirror. The process always proceeds from the edge of the disk. The budding figures which Schultz and Buding (14) and Habelmann (15) observed in immature blast cells would no doubt be dropshaped in living blood. Though their observations support my working hypothesis to a certain degree, they do not help to explain how the disk-shaped poikilocytes are formed (fig. 6 b—c).

Since this form of poikilocytosis evidently occurs in the process of new formation, it is suggested that it be called regenerative poikilocytosis.

In high-grade hypochromic anemia when the erythrocytes are extremely thin, poikilocytosis of another type is often seen. The blood cells are then pale and tattered. Some cells have two or more processes. On these occasions one always sees small hole-like defects or perforations in a number of otherwise faultless blood corpuscles. Some corpuscles show only a little circular perforation close inside the outer edge. Others have large projecting defects. Often the rounded outline of the cell is preserved only by a thin bridge of hemoglobin on one side (fig. 4 e). The perforations are best observed in stained smears. There the hemoglobin is seen collected around the hole in the same way as around the outer edge of the disk. This is important to remember when differentiating perforations from other pale spots occurring when the preparation is badly smeared or slowly dried. The perforations are also seen in living blood. Thus they cannot be artefacts. If a hemoglobin bridge bursts, a poikilocyte with two processes is the result (fig. 2). If several holes develop in one cell, the most

fantastic shapes may arise. Perforations of this kind naturally hinder the formation of spherocytes. If spherocytosis precedes hemolysis (18), this type of degeneration must be a hindrance to the normal disintegration of the erythrocytes.

Since the latter form of poikilocytosis apparently occurs in the peripheral blood, it is suggested that it be called degenerative poikilocytosis.

It is very important to be able to distinguish between regenerative and degenerative poikilocytosis when investigating the problem discussed in this paper. There are many other types of poikilocytosis in addition to those described, but they fall outside the sphere of this investigation. Undoubtedly many different factors can cause irregularity in the outline of the blood corpuscles.

Anisocytosis.

Under normal conditions the erythrocytes are very even in size, the diameter varying only within 2 or 3 μ . When there is a disorder in erythropoiesis, on the other hand, they vary greatly in size. In stained smears fragments down to the size of a thrombocyte are not uncommonly seen, and occasionally gigantic cells up to 20 μ in diameter. Anisocytosis and poikilocytosis are almost invariably associated.

It is commonly believed that the size of the erythrocyte is dependent upon the size of the erythroblast. Most hematologists emphasize that normocytes only come from normoblasts while megalocytes come from megaloblasts (4, 2, 19). Direct observations have led me to another opinion. Figure 6 a, for example, shows a megalocyte being constricted off from a small normoblast.

Normoblasts of greatly varying size are seen in normal marrow. In spite of this, their product, the non-nucleated erythrocytes, are surprisingly even in size. Furthermore, most of the normoblasts in normal marrow have not enough cytoplasm to make a normocyte. The marrow of pernicious anemia, on the other hand, is dominated by megaloblasts with enough cytoplasm to make giant cells.

The size of the blast cell, therefore, has probably no decisive influence on the size of the erythrocyte. The latter is probably determined by other factors.

Reticulocytosis.

All hematologists are agreed that reticulocytes are immature erythrocytes. They are a normal component of the blood and their number increases with increased formation of cells. They are demonstrable by staining with brilliant cresyl blue or other basic dyes. Supravital staining must be used for this purpose. In other words, the staining must be done on living blood. The substance reacting to the vital dye is distributed in a net within the blood corpuscle. Hence the name reticulocyte. This is not a good name, however, for it suggests the reticular endothelium (12). Before the staining the component reacting to the vital dye is diffusely distributed throughout the immature blood corpuscle. In ordinary smears stained according to Pappenheim it is manifested as polychromasia.

Heilmeyer (20) classified reticulocytes into four groups according to the degree of maturity. Group I is the most immature and contains large quantities of material reacting to vital stains. Group II and III show a progressive decrease in the amount, while group IV has only a few grains left.

Gripwall (21) showed that the reticulocytes have such a specific shape that they can be distinguished in living blood without any staining. He first observed them in a case of hemolytic icterus where they produced so-called hazy sedimentation. They lag behind in the sedimentation of citrated blood and give the plasma layer a diffusely reddish tinge. When they are finally precipitated a few hours later they are found on the surface of the blood cells. Gripwall found «hilus shapes», as he called them, in all his normal cases.

I (22) had already observed these peculiarly shaped erythrocytes in 1930. However, my observations were not published until later in conjunction with Gripwall's discovery. They fit like a missing link into the budding hypothesis (fig. 1).

They consist of non-nucleated blood corpuscles of a softly undulated shape which against the light appear to be pursed together at one area. I have previously called them «bundles», as this name gives the best idea of their appearance (fig. 3.). They vary in shape, however. Some are twisted like a propeller. According as the shape is simplified, the amount of substance reacting to vital dyes decreases. One is tempted to sort their

shapes into a sequence, the last stage of which is the normal, disk-shaped erythrocyte.

Since this type of cell is a well-defined stage between the normoblast and the mature, disk-shaped erythrocyte, it is suggested that it be called proerythrocyte.

The proerythrocyte is most easily demonstrated in citrated blood. Because it has no tendency to aggregate it is seen lying free between the rouleaux of blood corpuscles. It is easily detected in smears because of the pursing of its outline and its polychromatic staining. In supravital staining it is seen as a reticulocyte of groups I or II or sometimes III. Group IV is disk-shaped. The proerythrocyte is of slightly larger diameter than the mature erythrocyte in smears. Because of its slightly spherical form it appears slightly smaller in living blood.

In excessively active erythropoiesis, such as occurs after a large hemorrhage, the number of proerythrocytes is greatly increased. The greatest increase is seen in hemolytic icterus and in the first stage of remission in pernicious anemia, thus on the same occasions as the greatest reticulocytosis.

Proerythrocytes are much more numerous in the marrow than in the blood (23, 24). On increased erythropoiesis, masses of these elements are seen in the sternal marrow. Because they have not the same tendency to aggregate as the mature erythrocytes, they are often unevenly distributed in supravital staining.

C. M. Plum (25) and R. Plum (26) showed that the maturation of the reticulocyte was favored by a specific hormone, the reticulocyte maturity hormone. Cattle, which lack reticulocytes in the blood, have normally a large quantity of this hormone. Guinea pigs, whose blood contains an abundance of reticulocytes, have only a small quantity.

Erythroblasts in Blood and Bone Marrow.

Most of the earlier observations concerning nucleated red blood corpuscles have been made in circulating blood and dissecting-room material. The erythroblasts seen in the peripheral blood originate almost entirely from extramedullary erythropoiesis (2). The blood is not the right environment for erythroblasts. Thus degenerative changes, such as pyknotic or split nuclei, are often seen there. After-ripening of the cytoplasm also occurs, accounting

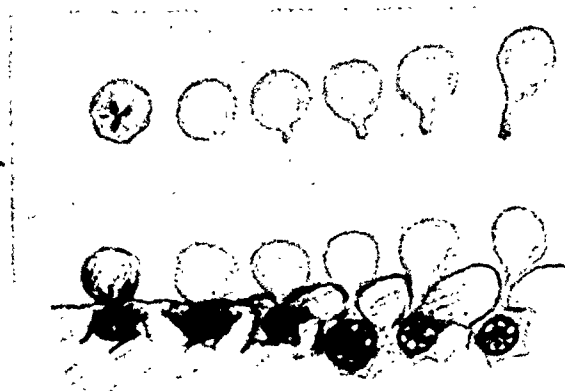


Fig. 1. Schematic hypothetical picture of the wall of a medullary sinus, showing how a regenerative poikilocyte develops (cp. fig. 4 a—d).



Fig. 2. Schematic hypothetical picture showing how a degenerative poikilocyte develops. The perforations increase in size and finally rupture the outer surface of the cell.

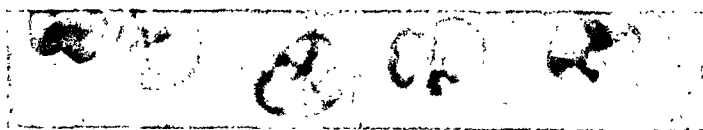


Fig. 3. Proerythrocytes (= unstained reticulocytes) in living blood. Photomicrograph $\times 1,000$. The cell at the extreme right is the most mature (cp. the cell at the extreme left in fig. 1).

BOSTRÖM: Non-Nucleated Erythrocytes.

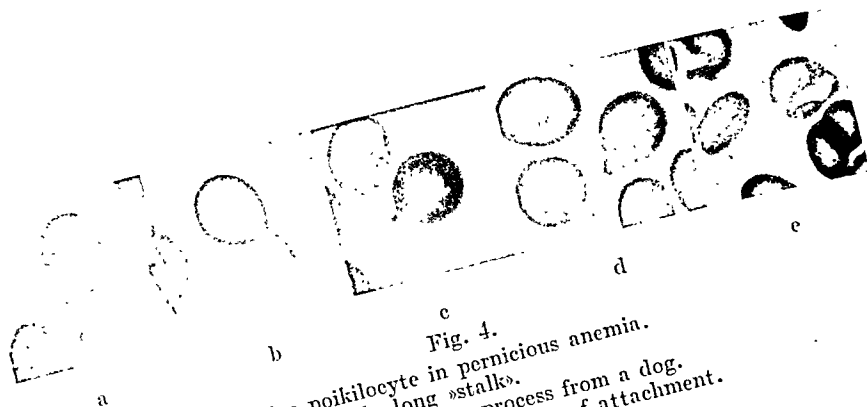


Fig. 4.

- a. Typical regenerative poikilocyte in pernicious anemia.
- b. Erythrocyte with unusually long «stalk».
- c. Normal erythrocyte with a tack-like process from a dog.
- d. Regenerative poikilocyte with a blunt place of attachment.
- e. Degenerative poikilocytes in hypochromic anemia.

Photomicrographs $\times 1,000$.

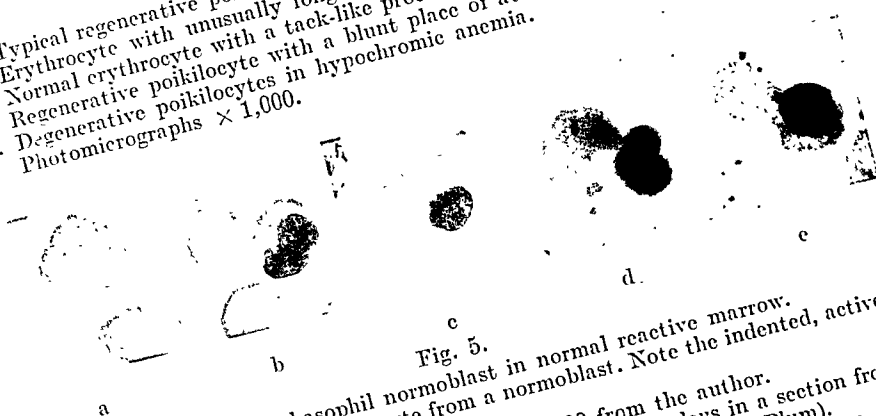


Fig. 5.

- a. Pseudopod from a basophil normoblast in normal reactive marrow.
- b. Detachment of a proerythrocyte from a normoblast. Note the indented, active nucleus.
- c. Mitroid normoblast. Photomicrograph $\times 1,000$ from the author.
- d. Detachment of a proerythrocyte from a normoblast nucleus in a section from a rat embryo. Note the indented, active nucleus $\times 1,200$ (from Plum).
- e. Budding off of an erythrocyte from a normoblast in vitro (from Plum). Photomicrograph $\times 1,500$.

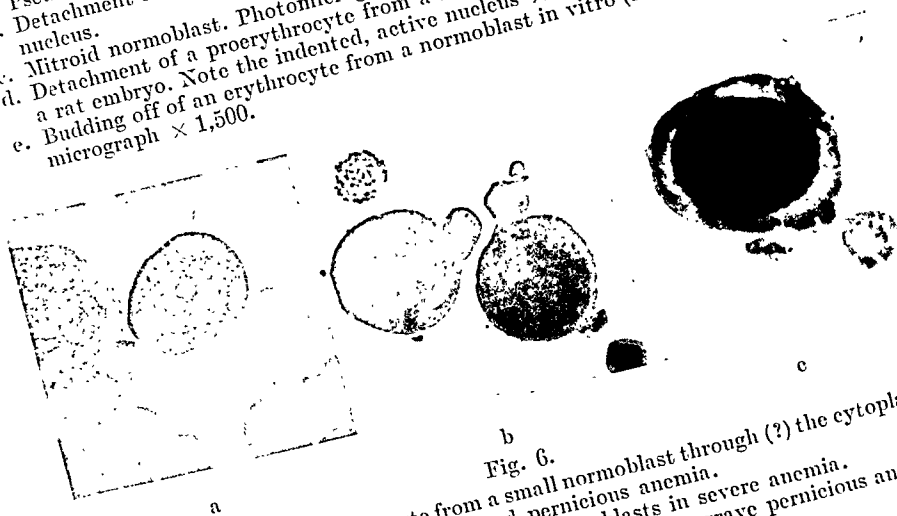


Fig. 6.

- a. Detachment of a megalocyte from a small normoblast through (?) the cytoplasm of a reticular cell in uncompensated pernicious anemia.
- b. Budding off of protoplasm from proerythroblasts in severe anemia.
- c. Budding off of protoplasm from promegaloblasts in grave pernicious anemia.

Photomicrographs $\times 1,000$.



Fig. 7.

- a. Nuclear contractions in intense erythropoiesis.
- b. Normoblast with a contracting nucleus and a giant cytoplasm, showing the great capacity of this type of cell to form hemoglobin-holding cytoplasm. The cytoplasm of this cell could make about ten proerythrocytes. It was taken from a case of pernicious anemia during the process of normalization.
- c. Normoblast syncytium during normalization in pernicious anemia (same case as in b.) Photomicrographs $\times 1,000$.

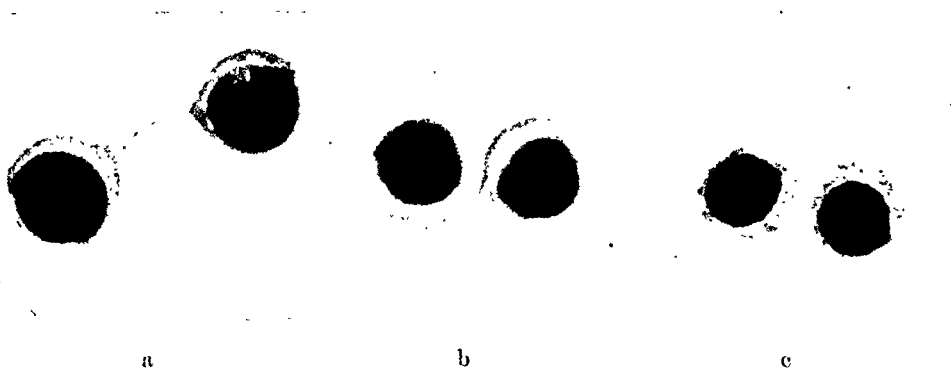


Fig. 8.

- a—c. Nuclear threads between erythroblast twins in sternal marrow. According to the author, Cabot's ring bodies in circulating blood are due to these threads. Photomicrographs $> 1,000$.



Fig. 9.

- a—d. Different types of Cabot's ring bodies in circulating blood.
- e. Normoblast with satellite granules in the cytoplasm. According to the author, Jolly bodies in erythrocytes are caused by these granules. Photomicrograph $\times 1,000$.

BOSTRÖM: Non-Nucleated Erythrocytes.

for the frequent observation of acidophil normoblasts in peripheral blood.

The rapid alteration after death has been described by Rohr and Hafter (27). They showed by series of sternal punctures in the dying and dead that the erythropoietic cells began to degenerate already during the death struggle. One minute after death twenty-four per cent of the normoblasts were changed and twenty minutes after seventy percent were karyorrhexic. Cellular studies carried out on autopsy material, therefore, should be regarded with criticism.

Not until sternal puncture according to Arinkin was accepted as a routine method, did the bone marrow become accessible to studies on a large scale. Though the fixed cells of the marrow are seldom included in the punctate and though the cells are wrested from their natural environment, sternal puncture has led to great progress in hematologic research.

Here I shall only discuss the erythropoietic part of the marrow. As customary, the term erythroblast is used to cover all forms of nucleated red cells concerned in erythropoiesis in mammals. Normally, the proerythroblast is the most immature form of these cells. It is a large cell with a pale nucleus and nucleoles. Its protoplasm stains deep blue. The next stage is the normoblasts with their characteristic nuclei. They fall into four groups, viz., large basophil, small basophil, polychromatophil and acidophil normoblasts. It should be noted that the acidophil normoblasts have the same color as the mature non-nucleated erythrocytes. The different groups of normoblasts are not sharply demarcated from one another. The cells always seem larger in thin smears than in thick ones. The staining is also more basophil in thick smears.

The small basophil and polychromatophil normoblasts dominate in normal marrow. Their nuclei are small and dense and stain a deep dark blue, showing up distinctly among the other elements of the marrow. They are generally considered pyknotic. It is true that no nuclear structure can be distinguished in thick smears. In thin ones, however, one sees a characteristic mosaic-like structure often showing a tendency to radial arrangement. The nucleus usually lies in the center of the cytoplasm. The cytoplasm varies a great deal. It is sometimes almost entirely lacking or confined to a narrow basophil fringe around the nucleus, making it difficult to distinguish the cells from small lympho-

cytes and reticular cells. Seldom is the cytoplasm sufficient to bring the cell to the size of a non-nucleated erythrocyte. The outlines are slack or lobulated and not so clearcut as those of the mature erythrocyte. The cytoplasm is cloudy and its staining reaction varies between purely basophil and polychromatophil. Acidophil normoblasts practically never appear in normal marrow.

In intense erythropoiesis in normal marrow, such as occurs after a large hemorrhage, excentrically situated nuclei is a common observation. The nucleus is not seldom seen lying more or less to one side of the cytoplasm. Nuclei without any cytoplasm are also observed. However, a «corona» of hemoglobin-free cytoplasm is generally seen around these «free» nuclei. When the erythropoiesis is extremely intense, the normoblast nuclei are often deuted or segmented (fig. 7 a). Howell-Jolly bodies are not seen in normal erythropoiesis. The smallest nuclei are 2 to 3 μ in diameter. Pseudopod-like buds of hemoglobin-containing cytoplasm are commonly found (fig. 5 a). Normoblasts of the same degree of maturity not seldom collect into clumps, into what appears to be fragments of large syncytia. These clumps of normoblasts are usually strewn over by reddish-violet granules of the kind seen in Ferrata cells. Ferrata cells with a highly granular flowing cytoplasm also occur.

The marrow picture in untreated pernicious anemia varies greatly from the normal. Large immature embryonic cells dominate. There is a great increase in mitosis. Only a few normal normoblasts are seen. On the other hand, there is a large increase in the number of acidophil normoblasts and normoblasts showing punctate basophilia. Their nuclei are often structureless and pyknotic, and the cells are probably dying or dead. Degenerated cells of all types also occur. Karyorrhxic nuclei, with granular satellites around an almost normal-sized nucleus, are also not infrequently present. I have observed fairly coarse threads connecting the nuclei of pairs of erythroblasts. Erythroblasts connected in this manner are always exactly alike (fig. 8 a—c). The thread is very graceful and can be traced all the way in to the nucleus. It generally stains red, but it may also be blue or colorless. Coarse nuclear threads between erythroblasts are only seen in grave disorders of erythropoiesis, though they are not specific of pernicious anemia. Fine threads between normoblasts is a relatively common observation. Segerdahl (28) has a picture of a set of megaloblast twins connected with a nuclear thread in

her thesis. She believed that the thread was composed of protoplasm and was the remainder of a cellular division. Budding off of cytoplasm from giant megaloblasts is seen in grave anemia (14) (fig. 6 c). Free bits of cytoplasm from other marrow cells are also seen (monocytes? Ferrata cells?). Only a few proerythrocytes are observed.

On the institution of specific treatment with liver preparations or folic acid, the marrow becomes normal in the course of a few days, the picture changing from megaloblastic to normoblastic. Large syncytium-like groups of normoblasts are a common observation during this process. Dense »blood islands» containing about a hundred fairly similarly sized normoblast nuclei, held together by a thin hemoglobin-free cytoplasm, are often seen (fig. 7 c). I observed several normoblasts with a giant polychromatic cytoplasm in one case (fig. 7 b). Large masses of proerythrocytes (reticulocytes) are always seen during the process of normalization. A relatively large number of acidophil normoblasts and erythroblasts with degenerated nuclei also occur.

Capillaries of the Bone Marrow.

Bizzozzero (29) pointed out as early as 1881 that more than half of the bone marrow consisted of blood vessels. According to Doan (6), there are many more vessels in the marrow than necessary for circulation, but a large number of them are collapsed.

Like other capillaries, the capillaries of the marrow have unicellular walls. When they dilate, however, their lumina become the size of that of a vein. When they widen to this extent, the wall must become extremely thin, if not actually lacking in some places. Doan (6), who calls these capillaries »intersinusoidal», believes that there is a fairly rapid variation in the width of the vessels. Whole groups of capillaries dilate or collapse at the same time.

Normal erythropoiesis takes place in spongy bone (chest bones, ribs, vertebrae). The hematopoietic parenchyma is thus enclosed in small cavities of bone.

Discussion.

My budding hypothesis, supported by the aforementioned facts, was first presented in a preliminary report in 1938 (22). It was described in more detail in a Swedish journal in 1941 (11). Al-

ready in 1938, I pointed out that it was very likely that one nucleated erythroblast gave rise to several non-nucleated erythrocytes by constricting off cytoplasm.

The first fact which supports my hypothesis is that the nucleated cells of the marrow are not enough to compensate for the continuous disintegration if it is assumed that every blood corpuscle has once had a nucleus. The figures tally better if a multiplier is inserted in the process.

Another thing which bears out the hypothesis is the difficulty in explaining where all the nuclei disappear. If it is only a question of budding off of a piece of protoplasm, the mother cell would be left intact, and no disintegration of nuclei would be necessary. This would explain why only normal-sized nuclei, if any, are found in normal erythropoiesis.

Excentrically situated nuclei and »detachment figures» may be phenomena which occur while the cytoplasm is in the process of detachment. One is not forced to assume that the nucleus is extruded, as Rohr (2) stated. The problem outlined by Schilling (5) at the beginning of this article is satisfactorily explained.

The question of the way in which the erythrocytes pass from the parenchyma of the bone marrow into the circulating blood solves itself, even though the vascular system is closed. Whether or not there is a continuous circulation of blood through the capillaries of the bone marrow is of no significance.

The controversy on whether erythropoiesis takes place within the vessels, as Sabin (7) believed, or outside the vessels, as Turnbull (10) believed, is settled. According to the budding hypothesis both are right, for it assumes that the first phase of erythropoiesis takes place extravascularly and the second phase intravascularly.

If, as I assume, the erythrocytes are formed by budding off from erythroblasts, a certain degree of anisocytosis is not surprising. What is more surprising is that the erythrocytes are so similar in size in normal blood. The budding hypothesis provides a simple explanation for the small fragmentary blood corpuscles as well as for the giant ones.

The regular occurrence of regenerative poikilocytosis in certain conditions, both in smears and living blood, belies any talk about artefacts. Edema being one of the characteristics of pernicious anemia, the assumption that the vascular walls are thickened in this disease (fig. 1) is not without reasonable foundation.

Working Hypothesis.

The theory of erythropoiesis now to be presented was suggested by my observations in circulating blood. The stalk-like processes on the edge of the erythrocyte disk seen so often in poikilocytosis are assumed to be real stalks by which the erythrocytes were once attached to the capillary wall. Figure 1 is a schematic representation of the formation of poikilocytes as I presume it to take place (11).

As seen from the figure, stalkless blood corpuscles would be formed when the capillary wall is extremely thin, as in normal circumstances. A thickened wall, or at any rate an abnormally long distance between the mother cell outside the vessel and the erythrocyte inside the vessel would require a bridge between them. The adherence of this link to the cell when it is detached would explain the stalk-like process of the poikilocyte. The process would thus be a malformation which reveals something of the mechanism of erythropoiesis.

This interpretation of the processes leads to a new interpretation both of the denucleation of blood corpuscles and of their manner of passage through the vascular wall.

By fitting in well-known morphologic facts with this working hypothesis, I shall now attempt to answer the question in the title of this article.

Poikilocytosis.

Irregularly shaped erythrocytes of all kinds are given the common name of poikilocytes. They may have the shape of pears, bullets, anvils, molars or other objects (4). Most hematologists believe that poikilocytes are formed secondarily in circulating blood under the influence of non-isotonic plasma. It is presumed that blood corpuscles of these irregular shapes are of inferior quality from the start (Ehrlich, Naegeli) (4). Schulten (12) stated that poikilocytes were artefacts produced when making the smears.

Many attempts have been made to explain poikilocytes. Ebbecke (13) considered that they were forerunners of spherocytes. Schultz and Buding (14) observed budding off of protoplasm from megaloblasts in a case of pernicious anemia, and believed that this explained the development of poikilocytes. Shortly after-

without any colloids, such as Ringer solution or physiologic salt solution. The membrane, which is not stretched when the cell is disk-shaped, becomes too large when it changes to the shape of a sphere. Thus it may be that it is the fluid pressure, acting equally on all sides, which compresses the blood corpuscle into the well-known crenated shape. Then it would not be a question of shrinking in the ordinary sense (17). Ebbecke (13) showed that disk-shaped blood corpuscles change to the crenated shape when they are exposed to strong pressure. There is then no change in the volume of the cells, only a re-arrangement of their contents. It is probably well known that the tendency to aggregate is inseparably connected with the disk shape (17). Neither proerythrocytes nor spherical crenated erythrocytes aggregate, and therefore lag behind in the sedimentation in citrated blood, separating from the other cells.

The accumulation of polychromatic hemoglobin-holding cytoplasm around normoblast nuclei which I observed in 1941 (11) supports the assumption that the normoblast is able to form many non-nucleated erythrocytes. Figure 7 b shows a normoblast with enough cytoplasm to make at least ten erythrocytes. There was obviously some hitch in the budding-off process in this case. If the formation of new cytoplasm keeps even pace with the budding off, the capacity of the normoblasts to form new cells is not observable. It is most likely that normally the normoblast sheds one erythroblast at a time. The normoblasts with giant cytoplasm observed the day before the peak of a crisis of reticulocytes, however, indicate that several blood corpuscles can be detached simultaneously. The detachment quotient probably adapts itself to the need of new cells.

Sternal puncture according to Arinkin was introduced in our clinic around 1930. My working hypothesis, which was based on observations in circulating blood, was then ready. The routine examinations of the bone marrow, however, have given it much valuable support.

The cytoplasm of the most mature erythroblasts of the normal marrow, the polychromatic normoblasts, has exactly the same appearance as the cytoplasm of the blood's most immature erythrocytes, the proerythrocytes. This is a strong indication that the proerythrocytes are detached from polychromatic normoblasts. I (32) have observed many stages of this budding off of cytoplasm in cases of intense erythropoiesis. Proerythrocytes

Cabot (30) reported that edema was present in about sixty-four per cent of his cases of pernicious anemia. Eppinger (31) observed thickening of the vascular walls of the spleen in pernicious anemia.

If the stalk-like processes develop as I assume, the non-nucleated blood corpuscle must be attached to the mother cell through the capillary wall long enough for the development of a real cellular membrane. During the time of maturation within the blood vessel, the blood corpuscle changes character. Its protoplasm changes from a gel to a sol while a genuine membrane is being formed. At the same time it changes in shape. When it is fully ripe, the erythrocyte breaks off and is washed away by the blood stream. It is then disk-shaped, has a tendency to aggregate and is purely acidophil on staining according to Pappenheim.

The mature, disk-shaped erythrocyte cannot pass through capillary walls. For the proerythrocyte (reticulocyte) to be able to do so, it must be of another nature. Its cloudy structure, non-specific, more or less spherical shape and tendency to basophilia indicate that it is more like other blood corpuscles. Like thrombocytes and leukocytes, therefore, it should be able to pass through the sinusoid wall. According to the hypothesis, the cell is normally connected with the mother cell throughout the process of maturation. If the proerythrocyte breaks away early, it is not certain that it is able to mature into a normal disk-shaped cell. It can become acidophil (after-ripening) but whether it can become disk-shaped is a question. Bundle-shaped proerythrocytes in circulating blood should perhaps be regarded as abortions, which immediately begin to disintegrate. During the reticulocyte crisis, there is no real increase in the total number of red cells. Not until the reticulocytosis begins to diminish is there any increase. The more mature the erythrocyte is before it loosens, the larger chance it should have of living a normal length of time.

The proerythrocyte probably acquires a surface membrane of some kind on its first contact with the blood. As the proerythrocyte is slightly larger than the mature erythrocyte, it is probable that its contents become more concentrated as it ripens. If so, a kind of negative pressure would result which may help to produce and maintain the biconcave disk shape. However, no doubt several different factors cooperate in the maintenance of the disk shape. The colloid content of the surrounding fluid obviously plays a large part. The disks soon change into spheres in fluid

plasm when it is constricted off. The satellite granules seen around abnormal normoblast nuclei correspond in my opinion to the Jolly bodies. Cabot's ring bodies may be formed when a loop of the nuclear threads previously described is included in the protoplasm. It is easy to understand how a ring, a figure of eight or a double eight could arise in a non-nucleated blood corpuscle by this means. Fragments of threads or skeins of threads within erythrocytes may be explained in the same manner (fig. 9). On the other hand, it is difficult to understand how the remains of a spherical membrane could form a double eight. Nuclear threads are never lacking in the marrow when Cabot's ring bodies occur in the circulating blood.

Another, and perhaps the strongest support for the older denucleation hypothesis is that the excretion of uric acid is increased during increased erythropoiesis. This increase has been noticed particularly during the normalization in pernicious anemia. Opsahl (34) described a case of pernicious anemia in which the patient had an attack of gout during a remission.

But not even this observation need belie the budding hypothesis. During erythropoietic equilibrium, only a small part of the bone marrow is active. The long bones contain only adipose marrow, and the short bones, too, contain no small quantities of fat. In pernicious anemia all the reserves are called upon. The entire bone marrow system is filled with immature erythropoietic cells. During normalization, dense blood islands of normoblasts are seen. This would indicate that it is the immature megaloblasts which, by repeated and rapid division, build up the normal normoblast tissue. In a short while the supply of normoblasts probably reaches an excess, and then a large amount of unnecessary immature cellular material is disintegrated. The fact that considerable disintegration occurs, even in the normal areas of formation, is seen from the abundance of acidophil cells and erythrocytes with degenerative nuclei in the sternal marrow throughout the process of normalization. A certain amount of physiologic disintegration of normoblast nuclei is also to be expected in greatly increased erythropoiesis.

Cellular movements are important to consider when reconstructing the formation of erythrocytes. What we see in smears and sections are only rigid camera shots. The movements escape our observation. Many still pictures, however, enable us to recon-

struct the movements to a certain extent. It was this way that Doan (6) exhibited the movements of capillaries.

The erythrocytes lacking their own power of movement, it has been assumed that they are pushed out by the pressure of new-growth. It may also be that the capillary movements are a dynamic factor. If whole groups of capillaries dilate at one time in a well-filled bone cavity, some of the tissue outside the vessels must be pressed into their lumina. There must be a considerable suction towards the lumina. The dilatory phase of the capillaries, therefore, probably coincides with the birth phase of all the blood corpuscles which have their blast cells in the parenchyma of the bone marrow. The penetration of the cells into the lumina is facilitated through the fact that when the vessels are dilated to the full, their walls must be lacking in some places. Leukocytes and thrombocytes wander out as free cells which immediately follow with the blood stream. The erythrocytes are retained by the capillary wall while they mature. Not until they are fully mature, biconcave disks are they washed away by the blood.

As mentioned before, indented and segmented nuclei are seen in intense erythropoiesis (fig. 7 a). These pictures may be interpreted as signs of nuclear movements. When constricted, the nucleus is small and dark, when expanded larger and lighter. Variations in the size and density of nuclei need not be dependent on their age. Intense contractions may lead to a picture reminiscent of segmentation, by constricting off particles of the nucleus. Pictures of this kind are generally interpreted as a sign of division of the nucleus, but they may also be due to reversible nuclear movements. The difficult question then arises of what is a sign of regeneration and what a sign of degeneration. It is possible that the rosette-like nuclear pictures often seen in circulating blood are caused by non-reversible nuclear movements in a non-physiologic environment. The cytoplasm in normoblasts with a rosette-like nucleus is usually acidophil. Everything points to it being a question of degeneration in this case.

Experiments recently published by the Danish workers Jacobsen and Plum (33) give further support to my budding hypothesis. The following is a short description of their work.

In order to study the erythropoiesis in rat embryos, serial sections were made of pregnant female rats. The blood formation

was followed from day to day. Up to the eleventh day, the blood was formed exclusively within the vessels. The erythrocytes of the blood were then nucleated. On the twelfth day after conception, faint signs of erythropoiesis in the liver were observed. The erythropoiesis in the liver reached its peak on the fifteenth, sixteenth and seventeenth day. On the seventeenth day there were traces of a primitive bone marrow. On the twentieth day distinct myelopoiesis could be observed in the marrow, but no erythropoiesis. At birth on the twenty-first day, the marrow showed intense erythropoiesis and from then on this activity increased every day.

The first non-nucleated erythrocytes appeared in the blood on the thirteenth day after conception. The liver had then become active. Jacobsen and Plum drew attention to the fact that the first non-nucleated blood corpuscles were formed from normoblasts of a special type which they called mitroid (fig. 5 c). The hemoglobin-containing cytoplasm in these cells first collects on one side of the nucleus like a cap, or mitre, and then loosens away. They described the process as a budding off of the nucleus. They thought that the nucleus disintegrated after it separated from the cytoplasm, but did not present any proof of this assumption. They believed that this type of erythropoiesis was normal only during a short period of the embryonic development. It was followed, they said, by the adult type in which the centroid normoblast, with the nucleus in the center of the cytoplasm, dominated. The question of how the centroid normoblasts lose their nucleus was left entirely open.

Jacobsen and Plum (33) also calculated the number of non-nucleated blood corpuscles formed per day. During the fourteenth and fifteenth day after conception, 10 non-nucleated cells per erythroblast were formed and during the seventeenth and eighteenth day about 60 per erythroblast. For comparison, they calculated the newgrowth in an adult rat and found that 35 to a hundred non-nucleated cells per erythroblast were formed each day. Jacobsen and Plum did not interpret their observations to mean that every normoblast delivers several erythrocytes. They assumed rather that there was a rapid proliferation of normoblasts. The fact that this would entail a mass death of nuclei was not discussed.

As I (32) have pointed out, the observations of Jacobsen and Plum in rat embryos support my hypothesis if one assumes from

them, not that the nucleus is detached from the cytoplasm, but that the cytoplasm is detached from the nucleus.

After these experiments, Plum (35) began with the culture of bone marrow *in vitro*. He used a modification of Osgood's (36) apparatus for this purpose. The main part of Plum's apparatus consisted of three tubes or cylinders inserted into one another. The two inner cylinders had semipermeable walls. The outer one was of glass. Bone marrow diluted with Ringer solution was put into the middle tube and air made to stream through the mixture. The smallest cylinder, which was lowered into this mixture, was filled with serum + a liver preparation, folic acid or the like, which was kept in circulation throughout the experiment. The outermost tube, which acted as a kidney, contained circulating Ringer solution. The whole apparatus was kept in a waterbath of 37° C.

Before the start, the number of nucleated and non-nucleated cells in the marrow mixture was counted. An hour after the experiment was begun, another sample was taken for counting in a counting chamber, and further samples were taken every hour from then on.

The number of nucleated blood corpuscles kept fairly constant, but the non-nucleated ones increased with about two per erythroblast per hour. Thus every erythroblast must give rise to about fifty erythrocytes per day.

In order to be able to observe the budding off in a microscope, Plum built a micro-apparatus using a Bürker counting chamber. This apparatus was built on the same principle as the large one. Nutritive fluid was made to stream past the marrow on one side and Ringer solution on the other. Plum observed that the cytoplasm collected on one side of the cell, bringing the nucleus into an excentric position, and then disengaged itself entirely. This phenomenon is seen in figure 5 c, taken from Plum. Plum (37) also filmed the course of events.

Plum's ingenious cultures have confirmed my budding hypothesis as regards a very important point. It is my hope that the hypothesis will stimulate experimental research on other questions in this field and thereby contribute to the final elucidation of the problem of the red blood corpuscles.

Summary.

The author presents a hypothesis, originally based on observations in circulating blood, that the red blood corpuscles are formed by the budding off of hemoglobin-holding cytoplasm from the erythroblasts in the marrow, not by denucleation as formerly believed. According to her hypothesis, one erythroblast can form several erythrocytes (fig. 1).

The stalk-like process often seen on the edge of a poikilocyte is assumed to be a malformation caused by the cell's passage through a thickened sinusoid wall. This deformity reveals something of the mechanism of erythropoiesis.

The budding hypothesis is supported by the following facts:

1) The erythroblasts of the marrow are not sufficient for the enormous amount of erythrocytes required each day if, as according to the denucleation theories, one erythroblast can only form one erythrocyte. The figures tally better if each erythroblast is assumed to form several erythrocytes.

2) It has been impossible to explain what becomes of all the «extruded» nuclei. The budding hypothesis explains why one only sees normally sized nuclei, if any at all.

3) Excentrically situated nuclei and «extrusion» figures can be explained as phenomena occurring during the process of budding.

4) The puzzling problem of the manner in which the erythrocytes leave the parenchyma of the bone marrow solves itself despite the fact that the vascular system is closed.

5) The contrary observations of Sabin and Turnbull are explained when it is assumed that the first phase of erythropoiesis takes place extravascularly and the second phase intravascularly.

6) Anisocytosis is explained as due to the detachment of differently sized pieces of protoplasm.

7) The reticulocytes fit nicely into the budding hypothesis. The most immature have a distinctive shape which is easily observed in living blood (fig. 3). The name proerythrocyte is suggested for this cell.

8) The cytoplasm of normoblasts in normal marrow looks exactly the same as that of the proerythrocytes. Both are polychromatophil.

9) Budding off of protoplasm from normoblasts is a common observation in intense erythropoiesis. Several microphotographs

NON-NUCLEATED ERYTHROCYTES.

showing the budding off of a blood corpuscle are attached (fig. 5 b—6 a).

10) Normoblasts with a giant cytoplasm are evidence that a normoblast is able to form more than one blood corpuscle (fig. 7 b).

11) Capillary movements according to Doan may be assumed to be the direct cause of the transport of the cells away from the parenchyma of the bone marrow.

12) Nuclear movements may be interpreted as a sign of intense erythropoiesis (fig. 7 a). An alternation between »systole» and »diastole» explains the sometimes densely and sometimes loosely packed nuclei.

13) Nuclear threads between erythroblasts have been demonstrated in the marrow in abnormal erythropoiesis (fig. 8 a—c). Normoblasts with satellite granules have also been seen. Cabot's ring bodies and Howell-Jolly bodies may be explained by the incorporation of these abnormal structures in the detached »psendopod».

14) Embryonic studies in rats by Jacobsen and Plum have shown that there is a separation between the nucleus and cytoplasm of the normoblast. They calculated that ten to a hundred non-nucleated erythrocytes per normoblast are formed each day.

15) Bone marrow cultures in vitro by Plum have shown that every erythroblast gives rise to about fifty erythrocytes per day. He observed the budding off of cytoplasm (fig. 5 d—e) in cultures in a counting chamber and was able to film the course of events.

Bibliography.

1. Schilling, V.: Physiologie der blutbildenden Org. in Handbuch d. norm. u. path. Phys. 730, 1928. — 2. Rohr, K.: Moderne Auffassung ber Abstammung u. Entwicklung der menschlichen Blutzellen, Schweiz. med. Wehnschr. 685, 1940. — 3. Leitner, St. J.: Die intravitale Knochenmarksuntersuchung, 1945. — 4. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, 1931. — 5. Schilling, v.: Die Pathologie der Erythrocyten, Med. Welt 130, 1938. — 6. Doan, C. A., Cunningham, R. S. and Sabin, F. R.: Experimental Studies on Origin and Maturation of Avian and Mammalian Red Blood Cells, Contrib. Embryolog. 361, 1925. — 7. Doan, C. A., Sabin, F. R.: Studies on the Origin of Blood Vessels and of Red Blood Corpuscles as Seen in Living Blastoderm of Chicks, Contrib. Embryolog. 272, 1920. — 8. Maximow, A.: Binde- gewebe und blutbildende Gewebe, in Handbuch der Mikr. Anat. des

- Menschen 232, 1927. — 10. Turnbull, H. M.: The anatomy of Erythropoiesis in Vaughan, J. M.: The Anaemias, 1936. — 11. Boström, L.: Beobachtungen über die Form der roten Blutkörperchen im norm. 1. anem. Blut, Nord. Med. 1319, 1941. — 12. Schulten, H.: Lehrbuch der klinischen Hämatologie, 1939. — 13. Ebbecke, U.: Erythrocyte und Spherocyten in ihrer Beziehung zur Hämolysen- und Senkungsgeschwindigkeit, Deutsche Med. Wehnschr. 64, 1938. — 14. Schultz, V. and Buding, A.: Über eigenartige Knochenmarksbefunde bei Pern. Ar. Deutsche Med. Wehnschr. 492, 1940. — 15. Habelmann, G.: D. Knochenmarksgenese der Poikilozyten, Klin. Wehnschr. 1135, 1940. — 16. Froboese, C.: Grundsätzliches zur histologischen Technik und Kuntsproduktfrage, Folia haemat. 309, 1941. — 17. Boström, L.: Sänka och skadade blodkroppar, Nord. med. Tidskr. 1407, 1935. Mikroskopische Kontrolle der S.R., Nord. Med., 1007, 1940. — 18. Bergenhem, B. and Fähræus, R.: Über spontane Hämolysenbildung im Blut. ... Ztsch. f. d. ges. exper. Med. 555, 1936. — 19. Nordenson, N. G.: Studies on Bone Marrow from Sternal Puncture, Acta Med. Scandinav. 1936, suppl. 78. — 20. Heilmeyer, L.: Handbuch allg. Hämat., 1933. — 21. Gripwall, E.: Zur Klinik und Pathologie des hereditären häm. Icterus, Acta med. Scandinav., 1938, suppl. 96. — 22. Boström, L.: Iakttagelser över röda blodkropparnas form, Nord. med. Tidskr. 590, 1938. — 23. Robertson, O. H.: Effect of Experimental Plethora on Blood Production, J. Exper. Med. 221, 1917. — 24. Schar-tum-Hansen, H.: Zur Morphologie des Sternalpunkt. bei Pern. An. und Makroblastom, Folia haemat. 58, 1937. — 25. Plum, C. M.: Under-sogelser over reticulocytmodningene in vitro, 1944. — 26. Plum, R.: Reticulocytmodningsindex under normale og patologiske forhold, 1947. — 27. Rohr, K. and Hafter, E.: Postmortale Veränderungen des menschlichen Knochenmarks, Folia haemat. 58, 1937. — 28. Segerdahl, E.: Über Sternalpunktionen, Acta med. Scandinav. 1935, suppl. 64. — 29. Bizzozzero, G., Torre, A. A.: in Virchows Arch. f. Anat. u. Physiol. 95, 1884. — 30. Cabot, R. C.: Ring Bodies (Nuclear Remnants?) in Anemic Blood, J. Med. Res. 9, 1903. — 31. Eppinger, H. (cit. Schilling:) in Handbuch d. norm. u. path. Physiol. 730, 1928. — 32. Boström, L.: Werden die roten Blutkörperchen der Erwachsenen durch Abschnürung von den Erythroblasten gebildet?, Nord. Med. 805, 1944. — 33. Jacobsen, E. and Plum, C. M.: Über die embryonale Produktion roter Blutkörperchen der Ratte, Folia haemat. 164, 1942. — 34. Opsahl, R.: Hematopoiesis and Endogenous Uric Acid, Acta med. Scandinav. 611, 1939. — 35. Plum, C. M.: Methods for Continuous Tissue Culture as Applied to Bone Marrow, Acta physiol. Scandinav. 260, 1946. — 36. Osgood, E. E. and Muscovitz, A. N.: Culture of Human Bone Marrow, J. A. M. A. 106: 1888, 1936. — 37. Plum, C. M.: Lecture by Congressus VI Cytologicus, Stockholm, 1947.
-

From the Medical Department of the Bergen City Hospital
(Norway). Chief: Gunnar Bøe, M. D.

The Value, as a Clinical Test, of the Titration of Aneurin in the Urine.

By

OLE STORSTEIN.¹

(Submitted for publication October 30, 1947.)

Introduction. Of late years efforts have been made to discover useful quantitative tests for vitamins in the organism, particularly with regard to the conditions under which they are excreted. This is necessary to a correct interpretation of a series of hypovitaminoses, as only quantitative tests can form the basis for rational treatment.

There is a profusion of reports of the favourable action of B1 on various conditions, mainly neurites and neuralgias. As it may be difficult clinically to determine the effect of treatment in these prolonged diseases, it is most important that we now possess a test which is useful in hospital.

Beri-beri is a rare disease in our latitudes. Formerly, a certain amount of beri-beri occurred in sailing ships on long voyages. As late as 1929, Nissen was able to report 52 cases of beri-beri on a Norwegian whaler in the Antarctic. The most recent case of beri-beri on board ship in Norway has been recorded by Bøe in 1939. In 1940 Vogt mentioned a case of beri-beri in a drunkard. Two cases of beri-beri caused by one-sided nutrition has been recorded by Vogt in 1945. In German concentration camps are reported cases of beri-beri (Thygesen, Rehfeldt).

It would seem to be chiefly certain polyneurites which, among the various diseases recorded as having been cured by B1, present a solid foundation for this treatment. In alcoholic polyneuritis,

¹ Hankeland sykehus, Bergen (Norway).

the supply of B1 is reduced, its reabsorption is defective, its excretion is increased, and the need for it is also increased. Thus there are here present all the pathogenetic factors accounting for the onset of an avitaminosis. Delirium tremens has been interpreted as an acute lesion of the tissues of the brain following lack of aneurin, and favourable results have been claimed for vitamin B1 treatment (Kloster).

The neuritis of pregnancy may be due to an increased need for and a decreased supply of B1 resulting from vomiting. The polyneuritis of prolonged diarrhoea is another example of »nutritional polyneuritis» (Strauss). In prolonged febrile diseases there is an increased need for, and a defective supply of aneurin. Most cases of beri-beri in the East make their first appearance during a febrile disease (Schretzenmayer).

There are very few accounts of aneurin analyses in B1 hypovitaminosis. Lehmann and Nielsen have worked with biological methods (phycomyces test) and have demonstrated a reduction of the aneurin content of the blood in a case of beri-beri. — Bang, employing the same method, has found the aneurin content of the blood to be reduced in cases of gastric achylia, chronic alcoholism, febrile disease, miseries, prolonged dietetic restrictions and diarrhoea. — Geill and Lindholm have found that the thiochrome method in Karrer and Kubli's modification and the rat-growth method give approximately the same results. They also find considerable larger excretion after parenteral than after peroral administration and reduced excretion in a few cases of diabetes. — Other investigators have worked with the thiochrome method in various modifications determining the aneurin urinary excretion, most of them after a tolerance dose. Reduced excretion of aneurin has been found in following diseases: alcoholic polyneuritis (Borson, McAlpine and Hills, Wassmann); diabetic polyneuritis, thyrotoxicosis, multiple sclerosis (Borson, Wassmann); ulcerating colitis (Borson); gastric achylia, uncompensated heart disease (Wassmann); Simmond's syndrome (Bierring and Iversen). — Crismer has found normal excretion in persons whose supply of calories was limited.

In this journal, Lindahl has given a survey of the available methods for the determination of vitamin B1 metabolism. He has put on record the results he has achieved by his investigations, and he concludes that the methods now in use are not suitable for clinical employment.

The method thus still contains certain sources of error. It is in the main the non-specific fluorescent substances in the urine which give trouble. They are not equally prominent in all the specimens of urine as table 1 shows. They are a very disturbing element in certain cases, and they give rise to a greenish-white fluorescence which renders an accurate reading difficult. In my experience it is in the main urines containing bile pigment with which it is difficult to work. As pointed out by Widenbauer, salicyl gives rise to marked blue fluorescence which is responsible for quite misleading figures. I have been able to demonstrate this point in a patient.

Wassmann has devised a tolerance test for determining the organism's B1 status. This test depends on the well-known fact (Harris and Wang, Widenbauer, Borson, Cowgill) that most of a parenteral injection is excreted in the course of the first hours. A delayed excretion does, however, occur in a few cases. In Wassmann's opinion a B1 hypovitaminosis is certainly present when a dose of 5 mg is given i. m., and less than 750 γ of it is excreted in the course of the first 24 hours.

My Own Material.

In the course of some nine months I have examined at the Medical Department of the Bergen City Hospital those patients who might conceivably be suffering from vitamin B1 deficiency as suspected on the pathogenetic lines I have indicated. My attention has been focussed in the main on polyneuritis. I also had occasion to investigate the B1 excretion in two cases of toxic diphtheria at the Epidemiological Department.

During this period I have examined 29 patients of whom 8 suffered from some form or other of polyneuritis. See table 2 where the results are indicated. The last 4 patients served as controls.

It will be seen that the excretion in the 24 hours varied so greatly that no conclusion could be drawn from it. The parenteral administration of 5 mg of B1 led to a rise of the excretion which in all but seven cases exceeded the limit of 750 γ put to it by Wassmann. All the four controls showed a rise of the excretion exceeding the minimum limit put to it.

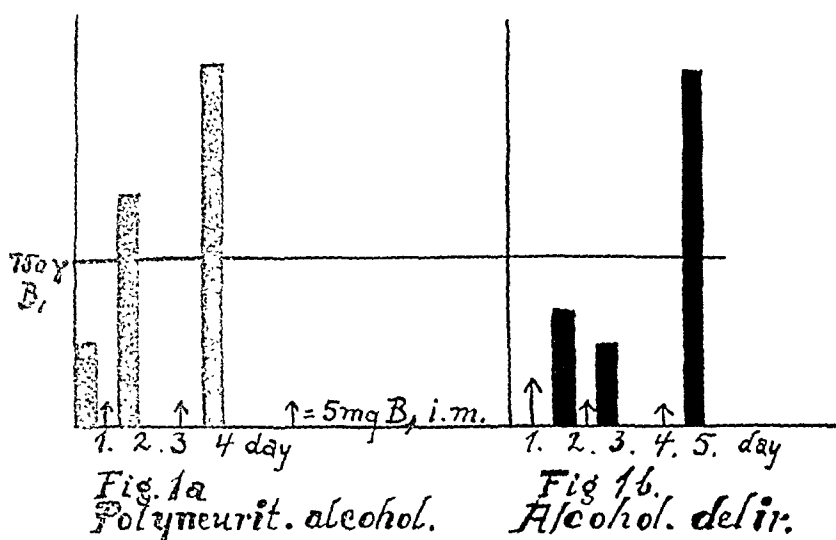
None of the eight patients suffering from polyneuritis showed

Table 2.

Pat. nr.	Sex	Age	Diagnosis	24 hours excretion before and after toleration test				
1.	M	62	Polyneur. alcohol.	360	1,056	1,650		
1.	»	»	Alcohol. delir.	495	350	1,600		
2.	»	55	Polyneur. alcohol.	1,700	1,176			
3.	»	43	» postdiphth.	436	952	980		
4.	»	30	» » ..	280	800			
5.	»	37	Polyneuritis	744	1,404	1,224		
6.	»	28	»		4,165			
7.	»	72	»	221	1,120			
8.	»	34			2,040			
9.	»	65	Parkinsonism	910	330	650	1,295	
10.	»	42	Epilepsy	270	721			
11.	F	51	Multiple sclerosis	165	198	850		
12.	M	30	» »		2,340			
13.	»	34	Dystr. muscul. progr. ..	72	2,295			
14.	»	25	Toxic diphtheria	208	756			
15.	»	30	» »		1,300			
16.	»	69	Myodegen. cordis	144	440	360	425	2,088
17.	»	45	Febris rheumat.	814	1,428	1,768		
18.	F	30	Diab. Emesis gravid. ..	780	1,980			
18.	»	»	14 days later		1,496			
19.	M	60	Pellagra		840			
20.	F	36	Hodgk. disease. Sprue		390	2,720		
21.	»	37	Thyreotoxicosis		1,425			
22.	»	60	»		2,250			
23.	M	47	Achylia	350	520			
24.	»	45	Ulcus duodeni	180	540			
25.	»	53	Enteritis acuta		2,080			
26.	»	51	Dyspepsia	495	780			
27.	»	82	Cancer prostatae	378	1,500			
28.	F	27	Cystitis		1,440			
29.	M	43	Pneumonia		1,530			

an excretion under the minimum limit, the one exception being the patient nr 1, fig. 1 a and b.

This patient was a casual labourer, aged 62, who had been admitted to the hospital nine times since 1923, on every occasion for chronic alcoholism. During his last stay in hospital in 1942, he suffered from a polyneuritis for which he was treated with B1. He was considerably better when discharged from hospital 3 weeks later. On his re-admission to hospital Sept. 6, 1943, his sense of touch was somewhat reduced on the anterior surface of the legs below the knee. His legs were rather weak, but the patellar and Achilles reflexes were satisfactory. Titration showed good aneurin excretion, as indicated by fig. 1 a. He was discharged from the hospital after three weeks, but was re-admitted Dec. 13, 1943. He was now suffering from acute pneumonia with mental con-

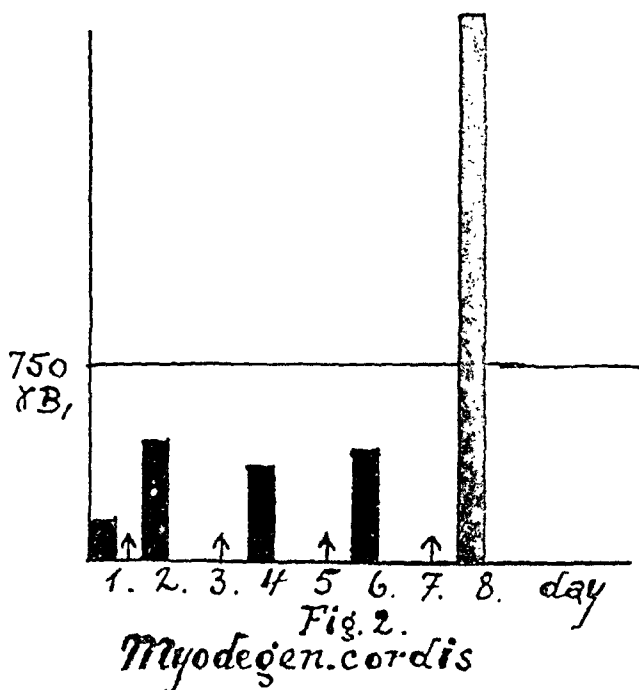


fusion, he was restless and lay picking at his blanket. Titration now showed a marked reduction of excretion which exceeded the minimum limit put to it only after three toleration doses, fig. 1 b. One is tempted to associate this reduced excretion with his beginning alcohol delirium. But it is also conceivable that his defective excretion in hospital on this occasion was due to his fever and insufficient food intake, as demonstrated by Wassmann. However, in the case of patient nr. 29, suffering from acute pneumonia, I could not find any reduction of the excretion. It is also possible that the administration of sulphapyridin may have influenced the B1 metabolism as pointed out by Jung. However, in the case of patient nr. 28, who suffered from cystitis and who was treated with sulphathiazol, no reduction of the excretion could be demonstrated.

Marked reduction of the excretion was also found in a case of degeneration of the heart muscle — patient nr. 16. He was 69 years old, and in 1939 had been operated on for stenosis of the pylorus by gastro-jejunostomy. For the past two years he had suffered from dyspnoea when at work, and during the last four weeks preceeding his admission to hospital there had been marked oedema of the legs below the knees. On admission to hospital Oct. 6, 1943 RR: 108/68. Eeg: myopathy? P: 56. Ewald: HCl/TA: 52/70. The severe oedema disappeared on a salt-free diet and rest in bed. Titration showed considerable reduction of the excretion of

aneurin, the minimum limit being reached and surpassed only after four tolerance doses, fig. 2.

Since his operation in 1939 this patient had been dieted, being limited only to fine bread. It may therefore be assumed that his food had contained but little aneurin. It is open to discussion whether his oedema and myopathy was due to lack of B1 or the diminished excretion of B1 was secondary to his heart failure.



The slow pulse, the satisfactory diastolic blood-pressure, and the beneficial therapeutic effect of rest in bed and a salt-free diet pointed against the diagnosis of beri-beri. The absence of neurological changes pointed in the same direction.

Patients nr. 23 and 24 suffering respectively from gastric ulcer and duodenal ulcer showed a reduced excretion after one tolerance test. The variations were, however, so small that they were within the margin of error.

Of two patients suffering from multiple sclerosis, one, nr. 11, showed reduced excretion after a tolerance test, fig. 3. This patient was a woman aged 51 whose disease had lasted seven to eight years. Treatment with B1 did not seem to have any remarkable effect on her condition.

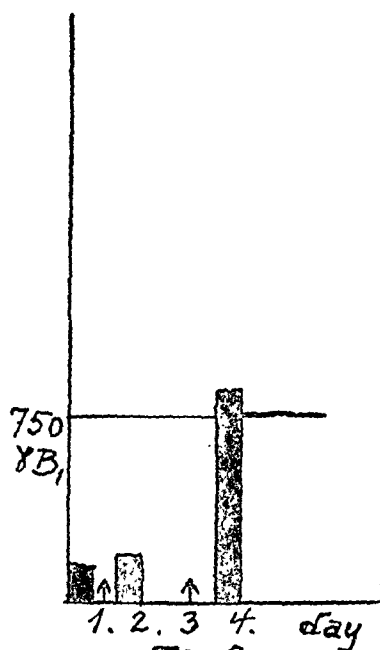


Fig. 3
multiple sclerosis

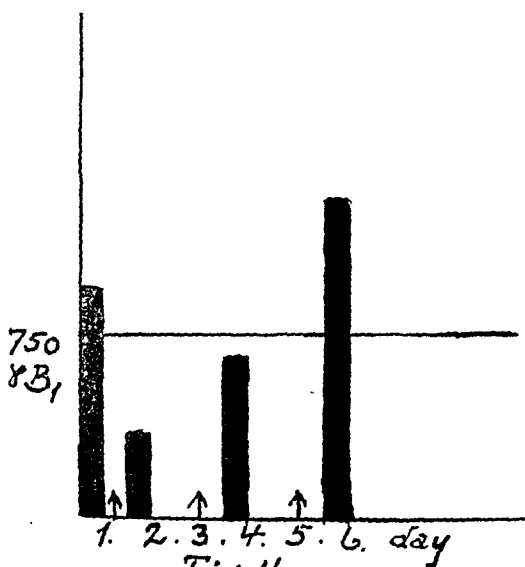


Fig. 4.
Parkinsonism

Patient nr. 9, suffering from Parkinsonism, showed considerable reduction of excretion after a tolerance test with B1, fig. 4. He was 65 years old, and for the past year he had been unfit for work on account of increasing weakness of arms and legs and stiffness on movement. All the groups of muscles showed considerable rigidity of the «cogwheel» type with great weakness. The

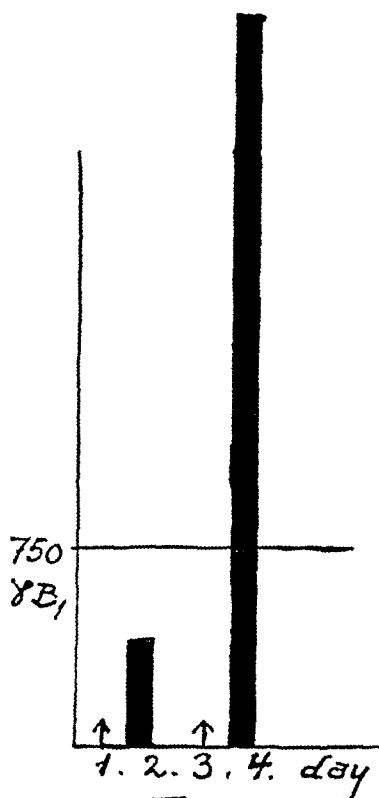


Fig. 5.
Hodgkins disease, Sprue

very scanty diuresis from which he suffered is a condition which leads, according to Widenbauer, to reduced excretion of aneurin. The figure shows that before a tolerance test his excretion within 24 hours was above the minimum limit being 750 γ . This sample was, however, very rich in carbonates and it was very difficult to read off the findings.

Patient nr. 20, who suffered from lymphogranulomatosis and sprue, showed reduced excretion after a tolerance test. She was 36 years old, and her malignant lymphogranulomatosis had lasted for two years. During the past half year she had suffered from

diarrhoea, and her light, somewhat bulky stools were seen under the microscope to contain droplets of fatty acid needles. Ehrström's test was faintly positive. Fig. 5.

A satisfactory excretion after tolerance tests with B1 was observed in the other patients who suffered from polyneurites, epilepsy, progressive muscle dystrophy, pellagra, diabetes with the vomiting of pregnancy, thyreotoxicosis and rheumatic fever.

Discussions.

The results obtained tally on the whole with those of Wassmann. The polyneurites, on which I have concentrated most of my attention, have been shown not to depend on a shortage of B1 in the cases I have examined. Even the alcoholic polyneurites did not, as might have been expected, show any reduction of the excretion of aneurin. We cannot, however, on the basis of a few titrations, dismiss the theory that a shortage of aneurin is responsible for the appearance of these polyneurites. For we must remember that alcoholic polyneuritis is a disease of long duration, and that these few titrations give us only a small cross-section of the patient's B1 status. These titrations give us no information concerning the patients condition at the first appearance of the polyneuritis. It may be assumed that an adequate diet during the days preceding the titrations may have corrected a moderate degree of aneurin deficiency should this have been present.

In the case of patient nr 1, who suffered from alcohol delirium, the titrations yielded information suggesting that aneurin deficiency may injure the nervous system. Both Borson and McAlpine and Hills, as well as Wassmann, have found the excretion of aneurin reduced in cases of alcoholic polyneuritis. Kloster has reported a case of alcohol delirium in which treatment with B1 had a good effect. Among the other forms of polyneuritis, that following diphtheria shows a normal excretion after a toleration test. Nor did the excretion show any reduction in toxic diphtheria, in correspondence with clinical tests at the Epidemiological Department by Bøe, where no difference could be found between one group treated with B1, and a second group, not treated. Experimental tests by Wassmann showed likewise no difference with treatment of diphtheric pareses in guinea pigs. Among other patients suffering from diseases of the nervous system there was

one epileptic and one case of progressive muscular dystrophy, the excretion of aneurin after a tolerance test was normal in both cases. Repeated tolerance tests in a case of diabetes with the vomiting of pregnancy and inadequate intake of food showed a normal excretion. There was no polyneuritis in this case either.

Contrary to the experience of Wassmann, I have found the excretion to be normal in two cases of thyreotoxicosis, but his patients were very thyreotoxic, to some extent on the verge of a thyreotoxic crisis. The excretion was normal in a case of pellagra, in which there was also no sign of aneurin deficiency. An antagonism between B1 and PP-factor has already been reported (Lehmann and Nielsen, Salvesen, Brændstrup). It may therefore be of interest to note that in this case tolerance testing with B1 led to a flare-up of the diarrhoea which was arrested by the parenteral administration of a complex B preparation (Storstein).

The material here presented is not large, but my task has been to gain an impression as to the reliability of the thiochrome method and its suitability for use in hospital. I have also sought evidence of B1 deficiency in diseases in which theoretical considerations made such a deficiency likely.

Summary.

A modification of the thiochrome method has been employed in the investigation of the excretion of aneurin before and after a tolerance test with 5 mg of aneurin given by intramuscular injection with a view to ascertaining the suitability of this method in hospital practice. Tolerance tests with aneurin were carried out on 29 patients in whom one might expect to find vitamin B1 deficiency. The tolerance tests showed an excretion of aneurin under 750 γ in the 24 hours after 5 mg of aneurin i. m. in cases of alcoholism, multiple sclerosis, Parkinsonism, achylia, sprue and cardiac insufficiency with nutritional deficiency. This was not, however, the case in various forms of polyneuritis.

In the author's experience this method is too laborious to be employed in hospital as a routine measure. It also has a considerably margin of error.

But this study and others are necessary if rational vitamin B1 treatment is to stand on a sure foundation.

Literature.

Bang, H. O.: B1-vitaminstudier ved hjælp af phycomycesmetoden. Ref. Nordisk Medisin 1944: 23: 1545. — Bierring, E. & Iversen, M.: Nordisk Medisin 1943: 19: 1115. — Borson, H. J.: Annals of Internal Medicine 1940: 14: 1. — Brændstrup, P.: Ugeskrift f. Læger 1940: 102: 95. — Boe, G.: Nordisk Medisin 1940: 7: 1631. — Bøe, J.: Acta Med. Scand. 1947. — Cowgill, G. R. in: The Vitamins — a symposium. Chicago 1939. — Crismer, R.: Acta Biol. Belg. 1941: 1: 437. — Fürst, V. jun.: Tidsskr. f. Kjemii, Bergvesen og Metallurgi. 1942: 4: 39. — Fürst, V. jun.: Personal communication. — Geil, T. & Lindholm, H.: Acta Med. Scand. 1946: 124: 522. — Jung, A.: ref. Wassmann. — Kloster, J.: Norsk Magazin f. Lægevidenskapen 1938: 99: 1301. — Lehmann, J. & Nielsen, H. E.: Nordisk Medisin 1939: 1: 289. — Lindahl, O.: Acta Med. Scand. 1943: 115: 485. — McAlpine & Hills: Quarterly Journ. of Medicine 1941: 10: 31. — Nissen, J.: Tidsskr. f. d. norske Legefor. 1939: 49: 1170. — Rehfeldt, Aa.: Nordisk Medisin 1945: 28: 2016. — Salvesen, O.: Nordisk Medisin 1940: 5: 279. — Schretzenmayer, A.: Ergebn. d. Inn. Med. u. Kinderh.k. 1941: 60: 314. — Storstein, O.: Nordisk Medisin 1945: 27: 1405. — Strauss, M. B. in: The Vitamins — a symposium. Chicago 1939. — Thygesen, P.: Nordisk Medisin 1945: 28: 2009. — Vogt, E.: Nordisk Medisin 1940: 7: 1600. — Vogt, J. H.: Nordisk Medisin 1945: 26: 830. — Wang, Y. L. & Harris, L. J.: Biochemical Journal 1939: 33: 1356. — Wang, Y. L. & Harris, L. J.: Biochemical Journal 1941: 35: 1068. — Wassmann, K.: Nordisk Medisin 1941: 11: 2582. — Wassmann, K.: Undersøgelser over udskillelsen af B1-vitamin med urinen hos sunde og syge mennesker. København 1944. — Wassmann, K.: Acta Med. Scand. 1946: 124: 27. — Widenbauer, F.: Ergebn. d. Inn. Med. u. Kinderh.k. 1939: 57: 351.

From the University Medical Clinic B. of Rikshospitalet, Oslo.
(Chief: Professor H. A. Salvesen.)

The Effect of Blood Transfusions on the Kidney Function of Chronic Nephritis with Anemia.

By

HARALD A. SALVESEN.

(Submitted for publication November 24, 1947.)

This paper deals with a minor problem, but one which should be investigated both from scientific and practical-therapeutic reasons. Is the kidney insufficiency of chronic nephritis aggravated by the almost constantly accompanying and often very severe anemia? It would be reasonable to suppose from a physiological viewpoint, that the function of an organ like the kidney, which is so dependent on a high minute volume and for the tubules on a high oxygen supply, would be unfavourably influenced if the blood was anemic.

The anemia of chronic nephritis has lately been the subject of some interest, especially as to its pathogenesis. All authors agree, that the degree of the anemia has a close relationship to the impairment of the renal function and it seems reasonable to suppose that it is caused by the retention of toxic substances acting on the bone marrow. Townsend, Massie and Lyons (1937), however, although they also found a parallelism between the degree of anemia and the nitrogen retention, were unable on bone marrow puncture to demonstrate any apparent lack of active blood-forming tissue. They found that with increasing anemia there is a decrease in the gastric acidity and conclude that the anemia is due to deficiency of iron caused by insufficient absorption. Nordenson (1938) on the other hand concluded that the anemia of chronic nephritis is conditioned by reduced bone marrow function due to an incipient aplasia of the erythro-poetic system, a view

Table 1.

Exp. no.	Name sex age	Date	Hgb. %	Red cells mill.	Color index	White cells	Alkali re- serve m.eq.	Blood urea mg	Urea clear- ance %	Remarks
1	O. H. ♀ 51	1938								
		Nov. 29	44	2,400	0.92	5,400	23.6	158	6	<i>Chron. nephritis</i> Transf. Dec. 1, 7, 13 and 20
		Dec. 24	91	4,219	1.08	7,500		191	6	
2	Hj. B. ♀ 38	1945								
		Sept. 29	55	2,290	1.22	10,000	16.8	279	7.0	<i>Chron. nephritis</i> Transf. Oct. 4, 7, 12, 15 and 19
		Oct. 4						267	6.0	
		" 5	59	2,830	1.05	8,400	18.9	258	6.0	
		" 20	92	4,360	1.05	9,600	23.6	162	7.0	
3	G. T. ♀ 57	Oct. 31	64	2,890	1.12	2,100	27.5	266	6.5	<i>Polycystic kid- ney</i> Transf. Nov. 3, 6 and 12 ¹ Died Dec. 7. Autopsy
		Nov. 7	85	3,640	1.19	3,300		237	6.0	
		" 19	94	4,360	1.08	5,200	29.4	308	6.0	
		Dec. 1	88	4,250	1.05	4,300				
		" 6					21.2	353	0.44	
4	O. L. ♂ 41	1947								
		Febr. 5	68	2,810	1.21	7,100	18.7	217	7	<i>Chron. nephritis</i> Transf. Febr. 11, 14, 17 and 21
		" 10					19.1	210	6.8	
		" 25	94	3,760	1.25	9,900	22.1	190	7.2	
5	F. H. ♂ 57	Febr. 8	61	3,390	0.91	4,300		81	22	<i>Chron. nephritis</i> Transf. Febr. 12, 15 and 19
		" 20	97	4,250	1.14	7,200		92	21	
6	K. B. ♂ 28	June 17	60	2,780	1.09	3,500	15.0	275	6.8	<i>Chron. nephritis</i> Transf. June 19, 23 and 27
		" 28	94	4,210	1.12	6,600	20.0	289	6.3	
7	"	Sept. 20	56	2,480	1.14	10,700	14.2	264	6.5	Transf. Sept. 24 and Oct. 1
		Oct. 2	71	3,210	1.10	12,100		262	8.2	
8	Aa. L. ♀ 43	Oct. 10	77	3,360	1.15	4,800	23.7	113	17	<i>Chron. nephritis</i> Transf. Oct. 15 and 17
		" 13						107	15	
		" 20	105	4,500	1.16	4,600		124	16	
9	S. R. ♀ 40	Oct. 15	75	3,090	1.23	5,900		95	19	<i>Chron. nephritis</i> Transf. Oct. 22, 24 and 27
		" 21	79	3,300	1.20	3,500	21.2	107	13	
		" 28	84	3,850	1.09	5,700	20.3	201	12.4	
10	H. S. ♀ 45	1946								
		Jan. 10	65	2,880	1.12	7,300	13.3	252	5.7	<i>Chron. nephritis</i> Transf. Jan. 17, 20 and 24 Recovered
		" 15						261	5.0	
		" 21	82	3,340	1.24	7,800	11.9	337		
		" 25	93	4,400	1.06	14,100	5.8	459	2.2	
11	B. M. ♀ 58	1947								
		Sept. 17	49	1,760	1.53	5,500	8.2	174	7.0	<i>Chron. nephritis</i> Transf. Sept. 18, 20 and 22 Died Sept. 25. Autopsy
		" 21	67	2,680	1.26	5,300				
		" 23	83	3,290	1.28	6,200	5.9	293	2.3	

¹ The patient had been under constant alkali treatment before the transfusions.

All of the cases had marked kidney insufficiency before the transfusions with urea clearances varying from 22 down to 5.7 (average 10.4). The anemia was also mostly severe with hemoglobin percentages ranging from 75 down to 48 (average 61). In 8 of the experiments (1, 2, 3, 4, 5, 6, 8 and 9) the urea clearance remained unchanged, although there might be some variations in the urea levels of the blood in both directions. In experiment 7 on the patient K. B. there was a slight increase in the clearance value (from 6.5 to 8.2 %), but the blood urea was unchanged. But in the last two experiments (cases H. S. and B. M.) there was a pronounced aggravation. In exp. 10 the plasma urea increased from 252 mg% to 459 mg%, the clearance went down from 5.7 to 2.2 % and the alkali-reserve from 13.3 vol% to 5.8 vol%. The patient's life was thought to be in real danger, but was finally saved by alkali treatment and she is still alive 18 months afterwards.

In exp. 11 there was also an aggravation with an increase in urea from 174 to 293 mg%; the clearance went down from 7.0 to 2.3 and the alkali reserve from 8.2 to 5.9 mM and the patient died a couple of days after in spite of energetic alkali treatment.

Comment.

The experiments show clearly, that the kidney function does not improve, when the blood is brought up to normal values of hemoglobin and red cells by blood transfusions. On the contrary it may prove dangerous and even fatal as shown in the last two cases with rapid increase of blood urea and decrease in the clearance and alkali reserve. This seems to give some support to the theory of Jensen-Jerre that the anemia is beneficial to the organism in its effort to counteract the acidosis. In experiment 11 (case B. M.) the aggravation might be a coincidence and the patient might have died anyhow, as the kidneys on autopsy were found to be extremely small, but the patient was not very ill and without serious clinical signs of uremia before the transfusions, with only a moderate azotemia and a clearance of 7 %, but with a serious degree of acidosis measured by the alkali reserve. This acidosis should have been treated first. It is reasonable to suppose that the blood transfusions were responsible for the rapidly developing uremia. And in experiment 10 (case H. S.) it was obvious that the blood transfusions caused the aggravation;

in spite of the low clearance of 2.2 % and the extremely high blood urea of 459 mg%, the patient recovered and is still living 1½ year after with only a moderate azotemia (a little above 100 mg%). The observation that in both these cases the alkali reserve decreased as the hemoglobin content went up seems to favour the view of an antagonism, as mentioned above, but in both cases the anemia was slightly, respectively decidedly hyperchromic, which does not fit in with the theory of Jensen-Jerre. It may be of importance that in these two cases the acidosis was more severe than in the other patients. There was clinically no indication that the aggravation was caused by the mechanical burden placed upon the heart by the three blood transfusions given with intervals of from 2 to 4 days.

It may be too strongly put to conclude that blood transfusions always are contraindicated in nephritic patients with kidney insufficiency and anemia, but they are of little help and should not be employed if the acidosis is severe.

Summary.

To 10 cases of more or less severe kidney insufficiency (9 cases of chronic nephritis and 1 case of polycystic kidney), all of them with pronounced anemia, blood transfusions were given in 11 different experiments until the blood was normal or nearly normal. In no case was there any improvement of the kidney function, measured by the urea clearance test. In 2 cases there followed a rapid aggravation with a decrease in the clearance value and the alkali reserve and increase of the urea retention; and one of these patients died two days after the last transfusion.

It is concluded that the anemia of the chronic insufficient kidney plays no part in lowering the kidney function and its treatment by repeated blood transfusions is of little benefit and may even be dangerous if the acidosis is severe.

Bibliography.

Ashe, B.: *Arch. int. Med.* 1929, *44*, 506. — Faarup, C. and A. Söeborg-Ohlsen: *Nordisk Med.* 1941, *11*, 2680. — Jensen-Jerre, O.: *Nordisk Med.* 1942, *13*, 51. — Möller, E., J. F. Mc.Intosh and D. D. Van Slyke: *J. clin. Invest.* 1928, *6*, 427, 485. — Nordenson, N. G., *Fol. Haematolog.* 1938, *59*, 1. — Townsend, R., E. Massie and R. L. Lyons: *Am. J. Med. Sciences* 1937, *194*, 636. — Van Slyke, D. D.: *Physiol. Reviews* 1921, *1*, 141.

Aus der Bernischen Heilstätte für Tuberkulöse in Heiligenschwendi
(Schweiz).

Untersuchungen über die Kantharidenblasen- reaktion bei Tuberkulose.

Von

Priv. Doz. Dr. ST. J. LEITNER unter Mitarbeit von W. THALMANN.

(Beider Redaktion am 6. Oktober 1947 eingegangen.)

I.

Die Kantharidenblase wurde bereits von E. Neumann (1878) zur Untersuchung der Entzündungszellen herangezogen, seither wurden aber einschlägige Untersuchungen kaum mehr durchgeführt. O. Müller und Gänsslen (1923) schlossen aus der Blasenzeit auf Permeabilitätsstörungen der Gefässe und Ödembereitschaft, Thomas und Arnold berichteten über spezifische Reaktionen im Blaseninhalt. Alder und Zaruski untersuchten die Eiweissveränderungen im Blut und in Exsudaten, darunter auch im Kantharidenblaseninhalte, bei Tuberkulose; darauf kommen wir noch bei der Besprechung der eigenen Ergebnisse zurück. Marquees bestimmte den Zuckergehalt in der Kantharidenblase (1935) und fand, dass sich dieser vom Blutzucker unterscheidet, und zwar parallel zur Zunahme der Zellzahl abnimmt, weil die Zellen im abgeschlossenen Entzündungsraum die Fähigkeit zur Sauerstoffatmung und Glykolyse haben (letztere Eigenschaft besonders die absterbenden Zellen). Diese Methode wandte Trautwein bei Tuberkulösen an (1942) und stellte fest, dass die Entzündungsstärke an der Abnahme des Blutzuckers erkennbar ist. Eine Diagnose der Art des tbc. Prozesses lässt die Methode nach ihm nicht zu, dagegen berichtet er über eine Zunahme der Entzündungsstärke in 20 % der Fälle bei konservativer Kur, in 50 % der Fälle bei spezifischer und Goldbehandlung und in

63.2 % der Fälle bei Thytebanbehandlung (abgetötete Tuberkelbazillen nach G. Schröder), was er mit der Aktivierung des retikuloendothelialen Systems (RES) durch die Behandlung erklärt. Robert und Strehler führten jüngst (1945) interessante Untersuchungen über den Fermentgehalt (Diaminooxydase) in der Kantharidenblase durch und fanden, dass entzündliche Reize (Verbrennung, Dermatitis) zu einem vermehrten Diaminooxydasegehalt führen, während die Blasenflüssigkeit im Bereich vitiliginöser Hautbezirke weniger Diaminooxydase enthält als im Bereich der nicht vitiliginösen Haut desselben Patienten; auch im Blaseninhalt im Bereich der normal-blassen Haut stellten sie weniger Diaminooxydase fest, als im Bereich der normal pigmentierten Haut der gleichen Person.

Uns interessieren hier besonders die Untersuchungen von F. Kauffmann aus der v. Bergmann'schen Klinik, der 1926 in mehreren Arbeiten über die Zellzusammensetzung in der Kantharidenblase bei verschiedenen Krankheiten berichtete. Normalerweise setzt sich der Zellgehalt der Kantharidenblase aus 0—3.25 % eosinophilen Leukozyten, 1.5—8 % ungranulierten basophilen Zellen und 89—98 % neutrophilen Leukozyten zusammen. Die ungranulierten basophilen Zellen hält Kauffmann für Abkömmlinge des retikulo-endothelialen Systems (RES), auch die kleinen, von den Lymphozyten morphologisch nicht unterscheidbaren Formen, weil bei diesen, im Gegensatz zu den Blutlymphozyten, die keine Fressfähigkeit ausüben (Metschnikoff, Ribbert, Marchand, Naegeli, Komiya, Leitner), eine Phagozytenfunktion nachgewiesen werden konnte. Wir selbst konnten diese Angabe von Kauffmann bezüglich der kleinen basophilen Zellen im Phagozytoseversuch bestätigen. Bei akuten Krankheiten, z. B. bei der Pneumonie, fand Kauffmann in der initialen Fieberperiode eine zellarme, sulzige Blase, in der Krise eine zellreiche, eitrigke Blase, während in der Rekonvaleszenz die Reaktion unerschwellig war oder eine Blasenbildung überhaupt ausblieb. In einer Nachprüfung stellte unlängst Min-Shen-Li fest, dass diese Reaktionen auch bei mit Sulfapyridin behandelten Pneumonien vorhanden sind, dass also das Mittel die zellulären Abwehrreaktionen nicht unterdrückt. Bei Tuberkulose liegen nur Untersuchungen von Kauffmann selbst vor, der bei aktiver Tuberkulose eine Zellvermehrung beobachten konnte, und zwar bei exsudativen Tuberkulosen mehr auf Kosten der Neutrophilen, bei produktiven Lungenprozessen mehr auf Kosten der Lympho-

Method.

The method I have followed in the course of my investigations has differed somewhat from that of Lindahl, and I propose to give a short account of my findings.

My method is based on that of Jansen which has been modified by Wang and Harris and by Wassmann. A report by Fürst jun. has also helped me in my investigations. As only slight changes have been made in Wassmann's method, reference is made to his study for a complete account of his method.

I have, however, found it most convenient to employ the original preliminary test of Wang and Harris as Wassmann's preliminary test entailed the use of such large quantities of cerisulphate solution — up to 20 ml — that the work became tedious for this reason. More recently, Wassmann has informed me that he has been obliged to dilute the urine five times, and he urgently advises the employment of his preliminary test. With this method the fluorescence is read off by direct visual inspection in front of a quartz-mercury lamp (Philips HPW 120), and the fluorescence of the test is compared with that of a control solution in which the aneurin is not oxidized to thiochrome, and which should thus contain the non-specific fluorescent substances in the urine. I have found it convenient to work with a standard which contains 10 γ per ml, whereas Wassmann has worked with a standard of 1 γ per ml.

By this method and by the addition of known quantities of aneurin to the urine, I have obtained the following results (table 1). It will be seen that added aneurin has been recovered in quantities of 64 to 114 per cent. The result coincides in the main with those of Lindahl.

Table 1.

Aneurin added per 100 ml		Aneurin found	Recovery %
Urine I.	0	28	—
	7.2	34	83
	17.2	45	99
	27.2	58	91
Urine II.	0	55	—
	4.5	59	89
	14.5	72	85
	24.5	79	96
Urine III.	0	31	—
	17	44	77
	14	40	64
	7	39	114

Die Eosinophilen in der Kantharidenblase stammen aus dem Blute; wir konnten bei 5 Kranken eine deutliche Eosinophilie im Blut, im Sternalpunktat und in der Kantharidenblase nachweisen, was für die Herkunft der Eosinophilen des Entzündungsgebietes aus dem Knochenmark bzw. Blute spricht, wie wir darauf a. O. hinwiesen (s. Knochenmarksmonographie von Leitner). Es fiel uns aber auf, dass die Blaseneosinophilie keineswegs immer mit der Bluteosinophilie parallel ging, es war manchmal trotz deutlicher Bluteosinophilie keine Vermehrung der Eosinophilen im Blaseninhalt feststellbar, und umgekehrt, es fand sich manchmal eine Blaseneosinophilie bei normalen oder leicht erhöhten Blut-Eosinophilenzahlen. Es spielt also hier die eosinotaktische Fähigkeit der Blase bzw. des Entzündungsgebietes eine Rolle. Auch die Neutrophilen in der Blase stammen aus dem Blute her, wie wir das besonders deutlich in einem Falle von Pelger-Huet'scher Kernanomalie sahen, wo sowohl im Blute, als auch in der Blase die gleichen typischen Pelger-Zellen mit der Segmentierungshemmung und charakteristischer Chromatinstruktur zu beobachten waren.

II. Eigene Untersuchungen.

Wenn es zutrifft, dass es sich bei der Kantharidenblase um Gewebsreaktionen des RES handelt, können Untersuchungen über ihr Verhalten bei Tuberkulose von grossem Interesse sein, wissen wir doch, dass das RES bei Tuberkulose wahrscheinlich massgebend am Abwehrkampf beteiligt ist. Nach Sabin und Mitarbeitern, Boivin, Leitner u. a. findet die Antikörperbildung im RES statt; eigene Untersuchungen sprechen dafür, dass die Immunkörper (nach Sabin Immunglobuline) in den spezifisch veränderten RES-Zellen, in den Epitheloidzellen gebildet werden (2). Das ist vielleicht auch der Grund, weshalb rein produktive Tuberkulosen ohne Verkäsung als günstig anzusehen sind, wie wir das in reiner Form beim Morbus Boeck beobachten können (5).

Wir haben nun 1. morphologische Untersuchungen des Blaseninhaltes durchgeführt, wobei die Menge und Beschaffenheit der Blasenflüssigkeit vermerkt wurde. Ferner wurde bei der Mehrzahl unserer Kranken die Gesamtzellzahl (in der Blutkörperchenzählkammer) bestimmt, oder falls das nicht möglich war, an Hand des Ausstrichpräparates beurteilt. Das Hauptgewicht wurde auf die Auszählung des gefärbten Ausstrichpräparates gelegt. Anfangs

haben wir die Zellen auch in Nativpräparaten mikroskopisch untersucht, doch war dabei keine verwertbare Differenzierung möglich. Die morphologischen Untersuchungen erstrecken sich auf 221 Fälle, von denen viele im Laufe der Kur monatlich wiederholt untersucht wurden. Bei 111 Kranken wurde ausserdem der Gesamteiweissgehalt und das Albumin/Globulinverhältnis im Blutserum und im Blaseninhalt bestimmt. Die eiweisschemischen Untersuchungen wurden auch in Hinblick auf die heute geltende Auffassung durchgeführt, dass die Bluteiweisskörper im RES synthetisiert werden (Sabin, Heinlein, Wuhrmann, Leitner [1, 2] u. a.).

1. Methoden.

Die Kantharidenpflaster wurden in 4 cm² Grösse auf die Streckseite des Oberschenkels aufgelegt, nachdem die Klebefläche über der Flamme erwärmt und die Haut mit Benzin abgerieben wurde. Über dem Pflaster wurde ein steriler Tupfer locker befestigt. Nach 22 Stunden wurde der Tupfer abgenommen, das Pflaster vorsichtig abgehoben und die Grösse der Blase vermerkt. Sodann wurde die Blase mit einer sterilen Kanüle angestochen und der Inhalt in ein Zentrifugenglas hineingelassen. Die Menge und Farbe des Reizexsudates wurden notiert, dann die Flüssigkeit gut durchgemischt und die Gesamtzellzahl in der Neubauer'schen Zählkammer bestimmt. Ein Teil der Blasenflüssigkeit wurde für die Eiweissbestimmung aufgehoben. Der Rest wurde mit einem Tropfen isotonischer Natriumzitratlösung (3.8 %) vermischt und 2 Minuten lang zentrifugiert. Das Sediment wurde auf einen Objektträger ausgestrichen, getrocknet und gefärbt. Da die gleichmässige Verteilung der Zellen im Ausstrichpräparat durch die Gerinnselformung vereitelt wurde, gingen wir nach Abschluss der Eiweissuntersuchungen so vor, dass wir ins Zentrifugenröhrchen von vornherein einige Tropfen isotonische Natriumtitratlösung gebracht haben, und zwar liessen wir sie an der Wand abfliessen, an welcher die Blasenflüssigkeit ins Gläschen hineingelassen wurde. Derart konnte eine Gerinnselformung meist verhindert werden. Wenn das aber nicht möglich war, so wurde sowohl ein Teil des Gerinnsels, als auch das Zentrifugat separat ausgestrichen, gefärbt und das Auszählungsergebnis beider Stellen miteinander verglichen. Verwertet wurden dann die Mittelwerte. Wie Kauffmann selbst diese Fehlerquelle der Inhomogenität zu vermeiden gesucht hat, wissen wir nicht, weil sich diesbezüglich keine Angaben in seinen Arbeiten befinden. Beim grossen Unterschied des Gerinnselformungsbildes und desjenigen des Zentrifugats ist aber die Verarbeitung eines möglichst homogenen Materials notwendig. Da das Sediment beim Färben manchmal mit abgewaschen wurde, haben wir in einer Reihe von Fällen die Objektträger mit Eiweiss vorpräpariert; bei genügend langem Trocknen und kurzer Hitze-

fixation lässt sich aber das Abwaschen des Präparates so gut wie immer vermeiden. Wir haben folgende Färbungen vorgenommen:

1. Anfangs haben wir als Vorfärbung Jenner-May-Grünwald Lösung (eosinsäures Methylenblau) mit der gleichen Menge destillierten Wassers verdünnt angewandt und nach Kauffmann 10 Sekunden lang mit konzentrierter, wässriger Methylenblaulösung nachgefärbt. Diese Methode haben wir später verlassen und die Nachfärbung wegen der schöneren Kernfärbung mit verdünnter Giemsalösung (8 Tropfen auf 5 ml destilliertes Wasser) 3—10 Minuten lang vorgenommen.

2. Bei einer Reihe von Kranken wurden Phagozytoseversuche mit Staphylokokken vorgenommen. Die Färbung dieser Ausstriche erfolgte mit wässriger Methylenblaulösung nach Fixierung mit abs. Alkohol 10 Sekunden lang. Der Phagozytoseversuch wurde analog der von Leitner in anderen Arbeiten (4) zuletzt in der Monographie »Die intravitale Knochenmarksuntersuchung« (Basel. B. Schwabe, 1945) beschriebenen Technik ausgeführt: In die frisch entnommene Kantharidenblasenflüssigkeit wurde 1 Tropfen einer Aufschwemmung von Staphylokokkus pyogenes aureus eingebracht und das Röhrchen für 1 Stunde bei 37° in den Brutschrank gestellt. Nachher wurden die Ausstriche nach der schon geschilderten Methode angefertigt und die Färbung vorgenommen.

Diese Versuche ergaben, dass sich die Lymphohistiozyten an der Phagozytose beteiligen.

3. Bei einem Teil der Kranken wurde die *Peroxydasefärbung* nach Moschkowski ausgeführt, um die Frage der Oxydasepositivität der Lymphohistiozyten zu prüfen.

4. Bei einem Teil der Kranken wurde die *Methylgrün-Pyroninfärbung* vorgenommen, um die Frage der Zugehörigkeit der lymphozytenähnlichen Zellen zu den Lymphozyten zu prüfen.

Bei 111 Kranken wurden *Eiweissbestimmungen im Blut und in der Kantharidenblase* vorgenommen, wobei das Refraktometer von Pulfrich und das Viskosimeter von Hess angewandt wurde. Wir sind uns bewusst, dass die refraktometrisch-viskosimetrische Methode ihre Fehlerquellen hat und für den Gesamteiweissgehalt eher zu hohe Werte angibt. Da es uns aber auf den Vergleich ankam, waren die Werte doch brauchbar, umso mehr, als geringfügige Unterschiede für unsere Zwecke doch nicht verwertbar sind. Wir haben die *Gesamteiweisswerte* und das *Albumin/Globulinverhältnis* bestimmt, wie dies in klinischen Laboratorien üblich ist.

Auf die Technik weiterer Untersuchungen gehen wir noch bei der Besprechung der Ergebnisse ein, hier sei nur erwähnt, dass auch das Verhalten des Blaseninhaltes nach Beeinflussung des RES mittels *intravenöser Kongorotinjektion*, ferner nach Allergisierung durch *Tuberkulingaben* sowie nach Herabsetzung des Parasympathikotonus durch *Bellafolin* geprüft wurde.

Da Kauffmann der Untersuchung der Kantharidenblase eine gewisse Bedeutung für die Diagnose und Prognose der Lungentuberkulose beimass, haben wir bei der ersten Hälfte unserer Untersuchungen parallel zur Kantharidenblase immer eine Blutsenkung und ein Blut-

bild angefertigt und die Resultate mit denen der Blasenmethode verglichen. Um die normale Reaktionsweise kennen zu lernen, haben wir bei 10 Gesunden bzw. Patienten mit nicht mehr aktiver Tuberkulose den Kantharidenblaseninhalte untersucht.

2. Die Gesamtzellzahl.

Die Zellzahl steht in einer ziemlich festen Beziehung zur Durchsichtigkeit des Blaseninhaltes. Bei einer Zellzahl von 2,000—3,000 war die Blasenflüssigkeit hell-serös, durchsichtig, bei zellreichen Exsudaten war sie trüb und bei einer Zellzahl von 50,000—80,000 sehr trüb bis eitrig. Bei Tuberkulose gehören eitrig-exsudate zu den Ausnahmen, unter unseren Kranken befanden sich nur 2, bei denen die Blasenflüssigkeit vorübergehend einen eitrigen Charakter annahm. Bei den späteren Untersuchungen haben wir die Gesamtzellzahl nicht mehr bestimmt, weil man aus der Transparenz des Reizexsudates auf sie schliessen konnte und weil die Feststellung der genauen Zahl mit der Art des Krankheitsprozesses keinerlei Beziehung hatte. Im Nativ- und Ausstrichpräparat liess sich die Zellzahl überdies schätzen.

3. Morphologische Untersuchungen.

Viel wichtiger waren die Befunde bei den morphologischen Untersuchungen des fixierten Ausstrichpräparates. Zunächst schien es, dass die Auszählung von 200 Zellen nicht genügt, um einigermassen gleichmässige und verwertbare Resultate zu erhalten. Nach einiger Übung zeigte sich aber, dass die von verschiedenen Untersuchern vorgenommenen Auszählungen sehr gut übereinstimmende Zahlen ergaben. Wir unterschieden zwischen grossen und kleinen lymph-histiozytären Zellen (= ly.-hist. Z.), welche wohl nach den Phagozytoseversuchen die gleiche Funktion haben, deren Verteilung uns aber trotzdem von Interesse zu sein schien. Ferner haben wir auch die Zellen notiert, die das typische Aussehen der Monozyten hatten. Ob sie allerdings den Blutmonozyten entsprechen, erscheint uns fraglich, wahrscheinlich gehören sie zu den grossen Formen der ly.-hist. Z. Indessen ist es auch durchaus möglich, dass einige Blutmonozyten, ebenso wie die Neutrophilen und Eosinophilen, aus dem Blut in die Kantharidenblase gelangten.

Zur Entscheidung dieser Frage und der Frage der Zugehörigkeit der ly.-hist. Z. zum RES gaben uns die *Phagozytoseversuche*

und die *Peroxydasefärbung* Anhaltspunkte. Erstere Versuche ergaben, dass die kleinen und die grossen ly.-hist. Z., auch die monozytoiden, an der Phagozytose beteiligt sind. Die Peroxydasereaktion war bei den ly.-hist. Z. in der Regel negativ. Die Monozyten des Blutes geben mit der Moschkowski'schen Methode eine schwach positive Peroxydasereaktion, so dass der negative Ausfall der Peroxydasereaktion bei den ly.-hist. Z. gegen die Identität der monozytoiden Formen mit den Blutmonozyten

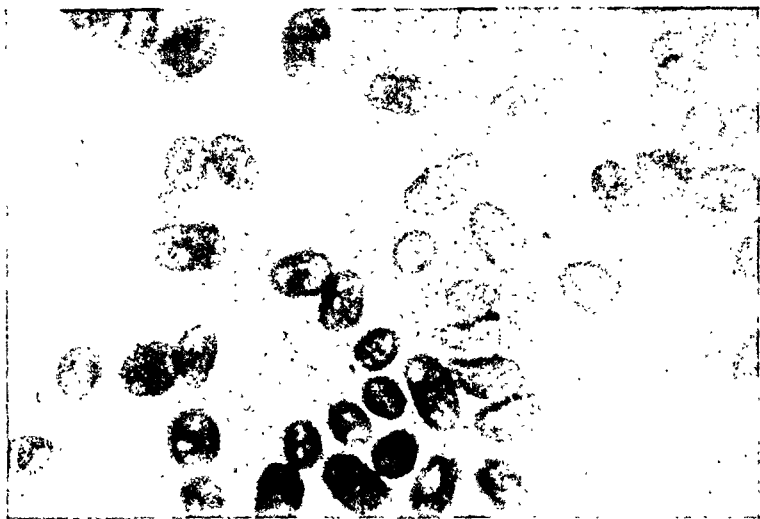


Abb. 1. Epithelzellverband in der Kantharidenblase (1200 \times).

spricht. Selten einmal war bei den grossen, rundkernigen ly.-hist. Z. eine Andeutung von einer Peroxydasefärbung zu sehen. Schliesslich sei noch erwähnt, dass auch bei Kranken mit starker Blutmonozytose keine Vermehrung der Monozyten in der Kantharidenblase feststellbar war.

So war bei einem Kranken mit Primärtuberkulose und Pleuritis der im Blutbild eine Monozytose von 27 $\frac{1}{3}$ % Monozyten aufwies, keine Vermehrung der Monozyten in der Kantharidenblase zu beobachten.

Während diese Untersuchungen, namentlich der Phagozytoseversuch, für die Zugehörigkeit der ly.-hist. Z. zum RES sprechen, konnte diese Deutung durch die *Methylgrün-Pyronin-Färbung* nicht gestützt werden, da sie sowohl bei den grossen, als auch vor allem bei den kleinen ly.-hist. Z. eine Rotfärbung des Protoplasmas zeigte. Es ist aber zweifelhaft, ob man aus diesem färberischen Verhalten auf die lymphozytär-plasmazelluläre Natur

der ly.-hist. Z. schliessen kann; es scheint vielmehr wahrscheinlich, dass die Rotfärbung auf die stark basophile Reaktion des Protoplasmas zurückzuführen ist, da, wie schon erwähnt wurde, die Lymphozyten nicht phagozytieren. Am wahrscheinlichsten ist es, dass die ly.-hist. Z. zum RES gehören und von den Uferzellen der Gefässe und vom Retikulum der Haut abstammen. Bei der Beurteilung der Präparate ist aber darauf zu achten, dass die ly.-hist. Zellen häufig eine grosse Ähnlichkeit mit Epithelzellen



Abb. 2. Lymphohistiozyten in der Kantharidenblase (1000 \times).

aufweisen, welche von der Blasenwand abschlüpfen. Besondere Vorsicht ist geboten, wenn solche Zellen in Verbänden vorkommen, wobei es sich meist um Epithelzellverbände handelt (*Abb. 1*). Bei den typischen Epithelzellen ist die Entscheidung leicht, namentlich, wenn zusammenhängende Verbände vorliegen. Manchmal, besonders bei Einzelzellen, ist dagegen die Entscheidung schwer und wir mussten die Diagnose oft offenlassen. Es ist aber nicht so, dass Epithelzellen in den meisten Fällen abschlüpfen und die Diagnose unsicher machen; vielmehr konnten wir häufig zellarme oder auch zellreichere Reizexsudate beobachten, wo keine einzige, mit Sicherheit als Epithelzelle identifizierbare Zelle feststellbar war. Die typischen Lymphohistiozyten sind in der Regel leicht zu erkennen. Bei den grossen Formen sind die Kerne der Zellen gross, das Protoplasma deutlich erkennbar (*Abb. 2*), während bei den kleinen Formen der Kern häufig die ganze Zelle einnimmt und der Protoplasmasaum, analog den Blutlympho-

zyten kaum oder gar nicht erkennbar ist. Bei den übrigen Zellen stösst die Diagnose auf keine Schwierigkeiten. Die Neutrophilen gelangen, wie schon erwähnt wurde, aus den Blutgefässen in die Kantharidenblase, ebenso die Eosinophilen, wenn auch ein Parallelismus zwischen Blut- und Blaseneosinophilie nicht besteht.

3 a. Die Eosinophilie.

Wir beginnen die zahlenmässige Wiedergabe unserer Befunde mit den Eosinophilen, bei denen die Verhältnisse am übersichtlichsten sind. Unter 221 Kranken fanden wir bei 36 eine Vermehrung der Eosinophilen im Reizexsudat von über 4 %. Bei den meisten dieser Kranken lag auch eine Bluteosinophilie vor, doch ging deren Grad nicht mit der Eosinophilenzahl im Blaseninhalt parallel, sondern wir konnten, wie schon erwähnt wurde, auch bei leicht erhöhter oder normaler Bluteosinophilenzahl eine Eosinophilie im Blaseninhalt finden. Zur Erklärung dieses Verhaltens muss eine Eosinotaxis im Bereich der Entzündung angenommen werden, die möglicherweise für allergische Vorgänge spricht. Immerhin konnten wir in je einem Falle von Morbus Boeck bzw. Asthma bronchiale die höchste Eosinophilenzahl in der Kantharidenblase feststellen, bei denen die Zahl der Bluteosinophilen 14 % bzw. 12 % betrug (Gesamtleukozytenzahl in normalen Grenzen). Die Tabelle 1 zeigt unsere Befunde. Dabei ist die Art des tuberkulösen Prozesses angegeben, um zu sehen, ob sich Schlüsse auf die Zugehörigkeit der Tuberkulose zu einer besonderen allergischen Lage ergeben.

Der Mechanismus der Heranziehung der Eosinophilen an den Ort der Entzündung ist noch nicht bekannt, wir wissen aber aus den experimentellen Untersuchungen von Kallós und Pagel, Walther u. a., dass bei im Tierversuch hervorgerufenen Anaphylaxien eine Ansammlung der Eosinophilen am Orte der Entzündung (Lunge) nachweisbar war, gleichgültig, ob die allergische Entzündung durch Histamin oder durch Sensibilisierung mit Eiweiss hervorgerufen wurde. Wir selbst konnten durch kutane Histamingaben (gemeinsame Untersuchungen mit Prof. Riesser) im Blut und im Knochenmark von Meerschweinchen nur eine geringfügige Eosinophilie feststellen, die dazu nicht einmal regelmässig zu beobachten war (vgl. Bericht des Schweiz. Forschungsinstitutes, Davos 1938 durch Prof. Roulet). Auch beim Menschen wurde bei allergischen Pneumonien, bei den eosinophi-

Tabelle 1.
(*Eosinophilie.*)

Diagnose	Zahl d. Fälle	Zahl der Eosinophilen in %
Spondylitis tbc.	5	12.5, 4.5, 10.5, 5, 4.5
Pleuritis exsudativa	2	11.5, 5
Peritonitis tbc.	1	23
Primärtuberkulose	3	9, 5, 6
Gelenktuberkulose	1	20
Hämato gene Lungentuberkulose ..	7	22, 15, 15, 14, 12.5, 9, 5
Miliartbe der Lungen	1	15.5
Iridozyklitis tbc.	3	12, 6, 5
Früh- und Sekundärinfiltrate ...	4	10, 9, 8, 7
Kavernöse Lungentuberkulose ...	6	17, 10, 7, 5.5, 5, 5
Morbus Boeck	1	41.5
Asthma bronchiale	1	32

len Lungeninfiltraten, eine reine eosinophile Pneumonie histologisch nachgewiesen (v. Meyenburg).

Es wäre von Interesse, wenn man aus den Ergebnissen auf die allergische Lage der Tuberkulose nach Ranke schliessen könnte. Wenn wir unsere Fälle daraufhin betrachten, so zeigt sich, dass unter den 36 Kranken 22 Prozesse aufweisen, die zum sekundären Stadium von Ranke gehören (intra- und extrapulmonale hämatogene Tuberkulosen). Da diese Zahlen noch nicht eindeutig sind, untersuchen wir das gleichzeitige Verhalten der Lymphohistiozyten. Von den 36 mit Eosinophilie einhergehenden Fällen wiesen 28 eine ly.-hist. Reaktion im Reizexsudat auf. Diese Zahl spricht für einen häufigen Parallelismus der ly.-hist. und Eosinophilenkurve. Während Kauffmann die neutrophile, eitrige Reaktion bei Pneumonie auf hochallergische Vorgänge zurückführt und mit dem Arthus-Phänomen in Beziehung bringt, sprechen diese Beobachtungen eher dafür, dass bei Tuberkulose, wenigstens in bestimmten Krankheitsphasen, die ly.-hist. Reaktion mit allergischen Vorgängen zu tun haben könnte. Die Zahl der ly.-hist. Z. schwankte bei den 28 Kranken zwischen 11 % und 54 %. Am höchsten waren die Prozentzahlen bei einer hämatogenen, doppelseitigen Lungentuberkulose, bei der es zu einer Kavernenbildung im rechten Oberfeld kam und die durch einen extrapleuralen Pnx günstig beeinflusst wurde (ly.-hist. Z. 54 %), ferner in einem Falle von exsudativer, subprimärer Pleuritis (38 %), in je einem Falle von Iridocyclitis und Spondylitis tbc (23 %) und in einem weiteren Falle von Spondylitis (20.5 %). Bei 2 kavernösen Tuberkulosen mit einer ly.-hist. Zahl von über

20 % handelte es sich um einen Mann mit multiplen, hämatogenen Lochkavernen, bei dem eine Pnx-Behandlung zu gutem Erfolg geführt hat und um eine 23j. Patientin mit doppelseitiger kavernöser Lungentbc, bei der ein Pnx links angelegt wurde (ly.-hist. Zahlen 29 bzw. 27.5 %). Beim ersten Fall ist die hämatogene Genese der Kavernen sehr wohl möglich. Es sei noch erwähnt, dass im Falle der Bronchustuberkulose, bei der bronchoskopisch eine Obturation des rechten Unterlappenbronchus mit tbc. Granulationsgewebe feststellbar war (bestätigt durch die histologische Untersuchung eines Probeexzisionsstückes) ein asthmatisch-dyspnoischer Zustand mit Atelektase des rechten Unterlappens bestand. Ob die Eosinophilie von 24 % durch den asthmatischen Zustand bedingt, also sekundär war, lässt sich nicht entscheiden; die ly.-hist. Zahl betrug 18 %.

Obschon eine Eosinophilie in der Kantharidenblase bei den zum sekundären Ranke'schen Stadium gehörigen Tuberkuloseformen häufiger feststellbar war, so sind die Befunde doch zu wenig eindeutig, als dass die Methode diagnostisch für die Annahme eines bestimmten Ranke'schen Stadiums verwertet werden könnte. Vielleicht vermag aber eine Eosinophilie im Blaseninhalt auch bei fehlender Bluteosinophilie etwas über eine allergische Reaktionsbereitschaft auszusagen.

3 b. Die neutrophile und lymphohistiozytäre Reaktion.

Eine Neutrophilie ist in der Kantharidenblase normal wenn die Zellzahl nicht erhöht ist. In der Krise akuter Krankheiten kommt es manchmal zu einer enormen Vermehrung der Zellen ganz auf Kosten der Neutrophilen. Eine lympho-histiozytäre Reaktion liegt aber in jedem Falle ausserhalb des Bereiches der Norm, wobei wir eine erhöhte Zahl erst bei mindestens über 10 % annehmen. Bei der Prüfung der Fälle auf eine neutrophile bzw. lymphohistiozytäre Reaktion hin sind zwei Fragen zu erörtern: 1. Die Frage ob sie für die Zugehörigkeit zu einem allergischen Stadium etwas aussagen und 2. ob sie für die Diagnose und Prognose der Tuberkulose im Sinne von Kauffmann verwertbar sind. Unter unseren Kranken wiesen 68 eine neutrophile und 78 eine lymphohistiozytäre Reaktion auf. Die Gruppierung der Fälle zeigt die folgende Tabelle 2.

Bei den anderen Kranken war eine normale Zellzusammensetzung der Kantharidenblase feststellbar, d. h. Neutrophilie ohne

Tabelle 2.

Diagnose	Neutrophile Reakt.	Ly.-hist. Reakt.
1. Knochen-Gelenktuberkulose	5	9
2. Peritonitis	3	6
3. Pleuritis exsudativa	6	2
4. Iridozyklitis tbc.	1	2
5. Lymphdrüsentuberkulose	—	2
6. Nierentbc	1	—
7. Hämatogene Aussaat der Lungen	3	16
8. Miliartuberkulose	—	1
9. Generalisierte verkäsende Tbc des ly.-Syst. .	—	1
10. Morbus Besnier-Boeck-Schaumann	—	4
11. Primärtuberkulose der Lunge	3	5
12. Primärkaverne	—	3
13. Früh- und Sekundärfiltr. der Lunge	7	6
14. Kavernöse Lungentuberkulose	38	20
15. Bronchustuberkulose	—	2
16. Keine aktive Tuberkulose	1	2
17. Asthma bronchiale	—	1
Insgesamt	68	78

Vermehrung der Gesamtzellzahl. Wenn wir die 146 Fälle der Tabelle 2 analysieren, so ergibt sich, dass eine ly.-hist. Reaktion bei den zum sekundären Ranke'schen Stadium gehörigen Prozessen häufiger zu beobachten war, als bei den Tertiärtuberkulosen. Besonders deutlich war das bei den 20 Kranken mit hämatogener Lungentuberkulose (inklusive Miliartbc), von denen 17 (85 %) eine ly.-hist. Reaktion und nur 3 eine neutrophile Reaktion aufwiesen. Die ersten 10 Krankheitsformen der Tabelle 2, bei denen die Zugehörigkeit zum sekundären Stadium von Ranke anzunehmen ist, waren 43 ly.-hist. und 19 neutrophile Reaktionen nachweisbar. Weniger deutlich ist dieses Verhalten bei den isolierten Organphthisen, bei den kavernösen Lungentuberkulosen, von denen 38 eine neutrophile und 20 eine ly.-hist. Reaktion zeigten. Auch wenn wir annehmen, dass einige kavernöse Tuberkulosen mit ly.-hist. Reaktion als hämatogene Kavernen anzusehen sind (bei 3 Kranken typische hämatogene Lochkavernen), ist die Verteilung der Fälle nicht so überzeugend, weil von 55 Fällen immer noch 17 eine ly.-hist. Reaktion aufweisen. Auch bei den Knochen- und Gelenktuberkulosen ist die Zahl der neutrophilen Reaktionen relativ hoch, wenn auch die ly.-hist. Reaktionen überwiegen. Das gleiche trifft auch auf die Peritonitiden zu, während sich bei den Pleuritiden sogar mehr neutrophile Reaktionen finden, als lymphohistiozytäre. Von den 8 Pleuri-

tiskranken gehörten 6 zu den subprimären Formen, die im Allgemeinen zum sekundären Ranke'schen Stadium gerechnet werden, obschon eine Generalisation, namentlich bei der Kontaktentstehung der Pleuritis, keineswegs immer gesichert erscheint (vgl. die Monographie über Primärtuberkulosen von Leitner und Steinmann⁶). Ebenso wenig aufschlussreich sind die Zahlen bei den Früh- und Sekundärinfiltraten, wo die neutrophilen und ly.-hist. Reaktionen sich die Waage halten. Zusammengefasst ergibt sich, dass die Generalisationsformen der Tuberkulose häufiger mit ly.-hist. Reaktion einhergehen, als die isolierten Organphthisen, wegen der vielen Ausnahmen ist aber eine zuverlässige Unterscheidung der Ranke'schen Stadien nicht möglich. Wir gehen dabei auf die Frage hier nicht ein, ob das Ranke'sche Schema eine grosse praktische Bedeutung hat, oder nicht; wenn wir vor Augen halten, dass Stadieninterpositionen bzw. rückläufige Stadiumwechsel nicht selten vorkommen, so behält die Ranke'sche Einteilung ihre Bedeutung, weil sie nicht nur das momentane klinische Bild, sondern das Tuberkulosegeschehen in seinem Ablauf in den Vordergrund stellt. Obschon unsere Befunde keine völlige Bestätigung der Kauffmann'schen Auffassung über die ly.-hist. Reaktion als Ausdruck der Ansprechbarkeit des RES erbracht haben, so verdienen die Feststellungen der häufigen ly.-hist. Reaktion bei den Generalisationsformen der Tuberkulose (Ranke II) doch unser Interesse, weil sie immunbiologische Vorgänge in ihrer Beziehung zum RES zu beleuchten vermögen.

3 c. Die Kantharidenblase im Verlauf der Tuberkulose.

Während etwaige Beziehungen der K. B. R. zur Ranke'schen Lehre Kauffmann nicht untersucht hat, stellte er die diagnostische Brauchbarkeit der Reaktion im Verlauf der Tuberkulose in den Vordergrund. Da dieser Frage eine grosse praktische Bedeutung zukommt, haben wir bei einer Reihe von Kranken alle 3—4 Wochen eine Kantharidenblase aufgelegt und die Blasenflüssigkeit in der üblichen Weise untersucht. Die Ergebnisse wurden mit dem klinischen Bild, Blutsenkung, Blutbild, bei einigen Kranken auch mit der Weltmann'schen Reaktion verglichen. Die Resultate können wir kurz fassen: von 44 Fällen ging die ly.-hist. Kurve in 29 mit der klinischen Entwicklung parallel, während sie dem klinischen Verlauf bei 15 Kranken nicht entsprach. Bei den 29 Kranken mit gleichsinniger Ent-

wicklung des Krankheitsverlaufes und der KBR war aber auch kein regelmässiger Parallelismus zwischen der Blasenreaktion und dem klinischen Verlauf feststellbar, indem die Zunahme der ly.-hist. Z. bei günstiger Entwicklung graduell nicht immer den anderen Untersuchungsmethoden entsprach; das gleiche gilt auch von der Abnahme der Lymphohistiozyten bei ungünstigem Verlauf. Bei den 15 Kranken war die Diskrepanz zwischen klinischer Entwicklung und KBR deutlich, während die Senkung hier nur bei 6 und das Blutbild bei 8 Kranken versagte. Als Beispiele seien Kurven von 6 Kranken gebracht. In der Abb. 3 sind 3 Kurven mit günstiger ly.-hist. Reaktion abgebildet, die dem klinischen Verlauf ganz entsprach; über die Fälle orientieren kurze Krankengeschichtenauszüge.

% Lymphohistiozyten

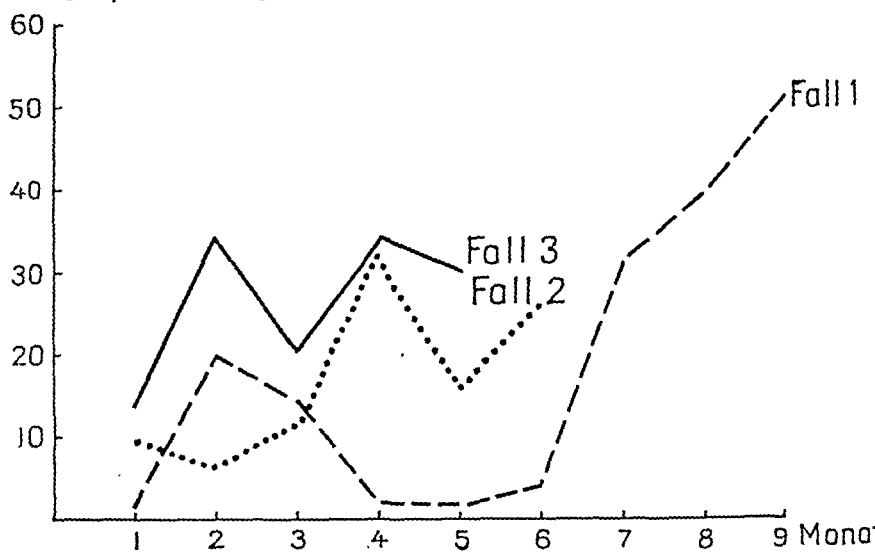


Abb. 3. Kurve 1. S. Text.

Fall 1. W. H. 19j. Haustochter wird mit einer Primärkaverne im rechten Unterfeld parakardial und exsud.-prod. Tbc im linken Mittel- und Unterfeld mit Kaverne eingewiesen (Kav. rechts 3.8×3.8 cm, links 2.2×2.8 cm). Sputum positiv (Gaffky + IV), Senkung 49/82 mm, Blutbild: L. 7,750 mit Lymphopenie von 14 % und Monozytose von 14 %. Nach doppelseitigem Pneumothorax Besserung, die Kaverne rechts blieb aber noch offen. Deshalb Phrenikusoperation rechts, die zu einem vollen Erfolg führte. Die ly.-hist. Kurve zeigte einen Anstieg von 1 auf 16 %, dann bei einem Rezidiv einen Rückgang auf 2 %; nach definitiver klinischer Besserung stieg die Lymphohistiozytenzahl

auf 52 %. Zu dieser Zeit Kaverne nicht nachweisbar, Sputum negativ. Senkung 10/38 mm. Blutbild normal.

Fall 2. M. R. 12j. Mädchen wurde mit einem noch nicht sehr lange zurückliegenden Primärinfekt (anamnestisch ca. 1 Jahr) rechts und hämatogenen Streuherden im linken Oberfeld eingewiesen. Bei konservativer Behandlung kam es zu einer sukzessiven Besserung, so dass das Kind inaktiviert entlassen werden konnte. Die ly.-hist. Kurve zeigte zunächst einen Rückgang von 9.5 auf 6 % und dann einen sukzessiven Anstieg auf 32 %; danach sank die ly.-hist. Zahl auf 16 %, ohne dass klinisch ein Grund hierfür gefunden werden konnte.

Fall 3. D. J. 22j. Mädchen wurde mit einer doppelseitigen mächtigen Hilusdrüenschwellung eingewiesen, die sich als die Hilusdrüsenform der epitheloidzelligen Granulomatose herausstellte (M. Boeck). Tuberkulin bis 1 : 100 ausgewertet negativ, Senkung 8/22 mm. Blutbild Leuko. 4050, davon Eos. 5, Mono. 10 %, sonst o. B. Während der Kur erhebliche Besserung, Rückbildung der Hilusdrüenschwellung und Beschwerdefreiheit. Die ly.-hist. Kurve zeigte einen Anstieg von 14 auf 33.5 % und blieb mit Schwankungen meist über 30 %.

Die nächsten 3 Kurven zeigen einen Abfall der ly.-hist. Zahlen, der bei 2 Kranken einer klinischen Verschlechterung entsprach, während bei der letzten Kranken trotz der Blasenreaktion eine Besserung eintrat.

Fall 4. K. H. 18j. Mädchen wurde mit einer Primärkaverne im rechten Unterfeld in die Heilstätte eingewiesen. Im Sputum reichlich TB, Senkung 27/53 mm. Blutbild: L. 8,700, davon B. 2, Eos. 5, Stab. 1. Segm. 50.5. Ly. 34.5. Mo. 6.5. Plasmaz. 0.5 %. Da bei konservativer Kur keine Besserung eintrat Pnx rechts. Obschon keine Adhäsionen bestanden, kam es nicht zu einem Kavernenkollaps. Pat. hatte ständig erhöhte Temperaturen. Pat. wurde nach einer 10monatigen Kur in ein Tieflandkrankenhaus verlegt, wo sie ihrem Leiden erlag. Die ly.-hist. Kurve zeigte nach Schwankungen einen Abfall von 34.5 auf 10.5 %, das Blutbild einen Rückgang der Lymphozytenzahl auf 10.5 % bei einer Monozytose von 12 %, Gewichtsabnahme von 52.0 auf 46.8 Kg.

Fall 5. S. H. 19j. Mädchen wurde mit einer prod.-exsud. Tbc des rechten Oberlappens mit mehreren Kavernen eingewiesen. Senkung 85/110 mm. Blutbild: L. 7,750, davon Eos. 2, Stabk. 6, Segm. 69, Ly. 20, Mo. 3 %. Pnx war wegen flächenhafter Adhäsionen nicht möglich. Es kam zur Streuung und Kavernisierung links, weshalb ein Pnx links angelegt werden musste. Nach Besserung links wurde eine Pneumolyse rechts vorgenommen, es war aber nur eine kleine Ablösung möglich, so dass der extrapleurale Pnx unwirksam war. Es wurde deshalb eine 6-Rippenplastik vorgenommen, die zur einer wesentlichen Besserung mit Verkleinerung der Kavernen führte, das Sputum blieb aber noch positiv. Die ly.-hist. Kurve zeigte einen Abfall von 38 auf 1 % und nach der Plastik einen Anstieg auf 12 %.

Fall 6. M. L. 34j. Hausfrau wurde mit einer prod.-kavernösen Tbc des linken Oberlappens eingewiesen. Da der Pnx infolge einer früheren

Pleuritis nicht möglich war, wurde eine Kavernendrainage vorgenommen, die zu einem ausgezeichneten Erfolg führte, der jetzt seit 4 Jahren anhält. Die Zahl der Lymphohistiozyten sank sukzessive von 42 auf 25 %. Die langjährige Senkung spricht für das Versagen der Kauffmann'schen Methode, allerdings hat auch die Sen-

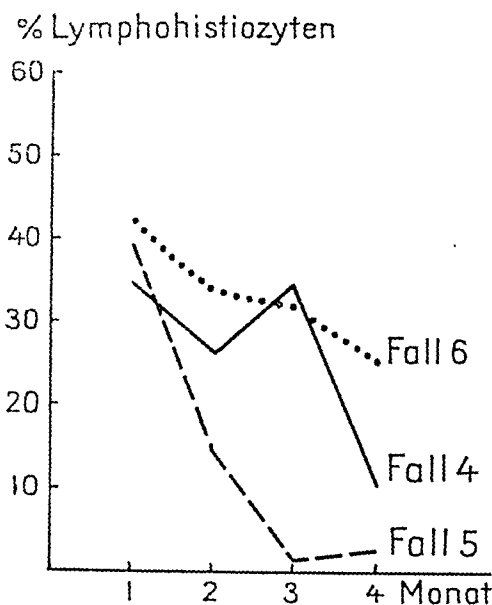


Abb. 4. Kurve 2. S. Text.

kung versagt, weil sie ebenfalls erhöhte Werte zeigt. Möglicherweise ist die Senkung auf eine leichte Suppuration im Stichkanal zurückzuführen (der Katheter wurde noch nicht entfernt). Ob die ly.-hist. Abfall ebenfalls darauf beruht, ist noch unsicher; das Blutbild blieb trotz erhöhter Senkung normal.

Ähnliche Kurven fanden wir wiederholt bei Kranken, bei denen der Krankheitsverlauf günstig war und mit dem Abfall der Lymphohistiozyten nicht im Einklang stand. Die zahlenmässigen Unterschiede waren manchmal gering, aber doch deutlich. Auch im umgekehrten Sinne hatten wir Versager, deutliche ly.-hist. Reaktion bei ungünstigen Fällen. Die stärkste solche Reaktion sahen wir bei einer Kranken mit der von uns beschriebenen generalisierten, verkäsenden Tbc des lymphatischen Systems¹ mit Tuberkulinanergie. Diese Beobachtung zeigt zugleich, dass die Auffassung von Kauffmann über das Ausbleiben jeder Reak-

¹ Leitner, St. J.: Acta thc. scand. 14, 277—323 (1940); Schweiz. med. Wschr. 1944, 8.

Histiozyten. Bei Besserung der Tuberkulose im Sinne einer produktiven Umwandlung kam es zunächst zu einer Vermehrung der Lymphohistiozyten, später, bei klinischer Heilung zu einer Normalisierung, mit Rückgang der Gesamtzellenzahl und Abnahme der Lymphohistiozyten. Da er die Lymphohistiozyten vom RES ableitet und die produktiven Veränderungen im Tuberkel ebenfalls durch Hyperplasie der retikuloendothelialen Zellen entstehen, führt er das Parallelgehen der granulomatösen Veränderungen in der Lunge und die lymphohistiozytäre Reaktion in der Kantharidenblase auf gleichartige Vorgänge im RES beider Organe zurück, wobei der Allergie eine grosse Rolle beigemessen wird. Kauffmann stützt sich dabei auf die Arbeiten von Rüsse, der im Tierversuch auf den gleichen entzündungserregenden Reiz einmal überwiegend neutrophile, anderemale eosinophile oder lymphoide Zellen im akuten Entzündungsgebiet erscheinen sah, und das formale Geschehen des Entzündungsgebietes für eins der feinsten Symptome der eingetretenen Allergie hielt.

In diesem Zusammenhang müssen wir uns zunächst vergegenwärtigen, um was für eine Reaktion es sich bei der Kantharidenblase überhaupt handelt. Gänsslen hält den Kantharidenblaseninhalte für Gewebsflüssigkeit, deren Beschaffenheit von den im Gewebe zirkulierenden Kolloiden und Kristalloiden abhängt; er schränkt diese Auffassung zugleich aber ein und nimmt an, dass die Blasenflüssigkeit zum Teil auch direkt aus dem Blute stammt. Kauffmann wies in Schnittpräparaten nach, dass der Blaseninhalt Schlüsse auf die Reaktion des Coriums zulässt, so dass die Kantharidenblase unspezifische Reaktionen des Hautorgans wieder spiegelt. Wir haben es demnach mit einer, wenn auch nicht reinen, Organreaktion zu tun. Wir wissen aber schon aus anderen Gebieten der inneren Medizin, dass biopsische Untersuchungen von Organpunktionen eine Bereicherung unserer klinischen Methoden darstellen, wie das uns die Ergebnisse der Sternal-, Lymphdrüsen-, Milz- und Leberpunktion zeigen. Als eine leicht reproduzierbare Reaktion einer örtlichen Entzündung der Haut war die Kantharidenblase für uns von Interesse. Es sei schon hier betont, dass die Blasenflüssigkeit eine andere zelluläre Reaktion aufwies, als die Tuberkulinpapeln, wie uns das systematische vergleichende Untersuchungen zeigten. Es sei ferner erwähnt, dass die Zellzusammensetzung in der Kantharidenblase nach unseren Befunden nicht vom Blutbild abhängt, eine Vermehrung der Lymphohistiozyten ging mit einer Blutlympho- oder Monozytose nicht parallel.

Wir sind auf diese Frage an anderer Stelle näher eingegangen (2). Ausserdem ergeben die eiweisschemischen Untersuchungen Anhaltspunkte für die Gefässpermeabilität, wie wir das an unserem Fall 7 sahen. Ferner haben Alder und Zaruski dem Eiweissbefund eine gewisse prognostische Bedeutung beigemessen, weil sie in 5 prognostisch ungünstigen Fällen einen niedrigeren Eiweissgehalt fanden, als in günstigen Fällen, was sie auf eine Verwässerung des Gewebes zurückführen.

Wir haben bei 111 Kranken eine Bestimmung des Eiweissgehaltes und des Albumin/Globulinverhältnisses im Blut und in der Kantharidenblasenflüssigkeit vorgenommen (Methode: Refrakto-Viskosimetrie nach Naegeli-Rohrer). Die Resultate sind weniger eindeutig und diagnostisch weniger verwertbar als die morphologischen Befunde. Die Ergebnisse zeigt die Tabelle 3, wobei wegen der Vergleichbarkeit der Resultate auch hier eine Gruppierung nach sekundärer und tertiärer Tbc durchgeführt wurde.

Tabelle 3.

(Eiweisswerte in der Kantharidenblase.)

Eiweiss in %	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	Summe
Sekundäre Tbc.	1	6	21	17	15	3	2	3	—	—	1	= 68
Tertiäre Tbc...	1	4	9	8	8	5	3	3	1	—	—	= 43
	2	10	30	25	23	8	5	6	1	—	1	= 111

Es ergaben sich also niedrige Werte im Blaseninhalt, die immer tiefer lagen als die Blutserumwerte. Da das Fibrinogen nur einen kleinen Teil der Bluteiweisskörper ausmacht, können die tiefen Werte im Reizexsudat nicht auf den Fibrinogenausfall zurückgeführt werden. Es erscheint vielmehr wahrscheinlich, dass sie auf der geringen Permeabilität der Gefässe für Eiweisskörper beruhen, sind diese doch normalerweise auch für die feindispersen Albumine nur wenig durchlässig; noch mehr gilt das für die grobdispersen Globuline. Bei sekundären Tuberkuloseformen hatten 53 Kranke einen Eiweissgehalt im Blaseninhalt von 5.5—6.5 %, zu denen noch 7 Kranke mit niedrigeren Werten kommen, so dass von 68 Kranken 60 (= 88.2 %) einen niedrigen Eiweissgehalt im Blaseninhalt aufwiesen. Bei den tertiären Formen war der Prozentsatz noch höher, von 43 Kranken hatten 30 (69.7 %) Werte zwischen 5.5 und 6.5 % oder weniger. Eine Unterscheidung der sekundären und tertiären Tuberkuloseformen ist auf Grund des Eiweissgehaltes demnach nicht möglich. Es

kommt offenbar nicht auf die Zugehörigkeit zu diesen Formen, sondern auf die Schwere der Tuberkulose an.

Bei Schwertuberkulösen fanden wir übereinstimmend mit Alder und Zaruski relativ häufig eine Zunahme der Globuline, wobei die Blutserumwerte aber immer höher waren. Eine Verwässerung des Gewebes (Reizexsudates) durch Wasseraustritt (herabgesetzter Eiweissgehalt) fand allerdings nicht immer statt, manchmal, wie im Fall 7, kam es zu einer relativen Albuminvermehrung, was mit der Permeabilität der Gefässe für die feindispersen Eiweisskörper zwanglos zu erklären ist. Für die diagnostisch-prognostische Beurteilung der Tuberkulose eignet sich die Eiweissbestimmung im Reizexsudat wegen der zeitraubenden Technik und schweren Deutbarkeit der Resultate nicht für die Praxis.

5. Das Verhalten des Kantharidenblase nach Tuberkulinisierung.

Da die Reaktion in der Kantharidenblase mit allergischen Vorgängen in Zusammenhang gebracht wurde, hat uns die Frage interessiert, ob eine Allergisierung durch Tuberkulin Veränderungen des Blaseninhaltes hervorzurufen vermag. Nach Untersuchung der üblichen Kantharidenblasenreaktion wurde 0.1 ccm einer Tuberkulinlösung von 1:10,000 (bei Kranken die früher auf Tuberkulin stark reagierten 1:100,000) intrakutan injiziert. Gleichzeitig und weitere 24 Stunden später wurden neue Pflaster angelegt und das Reizexsudat 22 bzw. 44 Stunden nach der Tuberkulinzufuhr untersucht. Gleichzeitig wurde die Stärke der Tuberkulinreaktion notiert. Da die 44-bzw. 48-Stunden Reaktion bei der Tuberkulose als die eigentliche spezifische Reaktion angesehen wird, während bei der 24 Stunden Reaktion unspezifische Komponenten mitspielen, ist der Spätreaktion die grössere Bedeutung beizumessen. Frühere Untersuchungen von v. Romberg, Otfried Müller und Brösamlen, Leitner (3) (1929) sprechen dafür, dass kleine intrakutane Tuberkulingaben sehr häufig eine Allgemeinwirkung haben, namentlich bei Tuberkulösen, wobei bestimmte Blutbildveränderungen eine allergisierende Wirkung annehmen lassen (O. Müller und Brösamlen, Leitner). Unsere Ergebnisse zeigt die Tabelle 4.

Ergänzend zu dieser Tabelle sei bemerkt, dass die Gesamtzellzahl in 10 von 14 Fällen zunahm, z. T., sogar erheblich. Während diese Zahlen recht eindeutig sind, zeigten die Eosinophilen keine

Tabelle 4.
(Die Kantharidenblase bei Tuberkulinisierung.)

Fall	Diagnose	Eosino- phile		Neutro- phile		Lympho- hist.		Eiweiss %		Alb/Glob.- Verhältn.	
		vor	nach	vor	nach	vor	nach	vor	nach	vor	nach
1. K. F. 33j.	Doppels. prod. Lungen- tbc.	12.5	2.0	76	87	11.5	11.0	5.9	6.2	70/30	53/45
2. G. J. 26j.	Pleuritis sicca.	0.5	0.5	95	89.5	4.5	10.0	5.4	5.9	55/45	70/30
3. Sch. E. 20j.	Doppels. Pleuritis ex- sud.	1.0	—	88	89.5	8.0	3.0	5.4	5.1	55/45	30/70
4. G. M. 31j.	Doppels. kavernöse Lungentbc.	—	3.5	93.5	89.5	6.0	7.0	5.1	5.7	30/70	50/50
5. E. M. 51j.	Doppels. kavernöse Lungentbc.	—	2.0	95.0	92.0	5.0	6.0	6.7	5.9	45/55	55/45
6. N. F. 40j.	Kavernöse Tbc li.	—	—	97.5	86.0	2.5	14.0	5.7	5.7	25/75	50/50
7. G. A. 21j.	Prod. Tbc links, Infil- trat rechts.	—	0.5	96.0	98.5	4.0	1.0	5.4	4.8	40/60	45/55
8. S. L. 38j.	Kavernöse Tbc re., Pneumothorax re.	2.5	2.0	57.5	91.0	40.0	7.0	5.6	5.9	65/35	60/40
9. F. E. 40j.	Doppels. kavernöse Lungentbc.	—	0.5	77.0	92.5	23.0	7.0	5.4	5.5	55/45	60/40
10. E. R. 31j.	Kavernöse Tbc li. Tho- rakoplastik.	—	1.0	58.0	83.5	39.0	15.5	5.4	5.5	55/45	60/40
11. B. H. 26j.	Kavernis. Infiltr. re. Pnx re.	1.0	1.0	95.0	97.0	4.0	2.0	4.7	5.7	30/70	40/60
12. Z. M. 21j.	Kavernöse Tbc re.	2.5	4.5	89.0	89.5	9.5	6.0	5.4	5.9	45/55	55/45
13. B. G. 20j.	Kavernis. Infiltr. li., Pnx li.	3.5	1.0	88.5	95.5	8.0	3.5	5.1	5.7	45/55	50/50
14. B. E. 23j.	Kavernöse Tbc li. Pnx li.	—	—	93.0	96.5	7.0	3.5	5.4	5.1	55/45	45/55

verwertbaren Veränderungen, bei 6 Kranken fand sich eine Zunahme, bei 6 eine Abnahme und bei 2 blieben die Eosinophilenzahlen unverändert. Klarere Verhältnisse wiesen die Neutrophilen auf, die in 10 von 14 Fällen zunahmen, immer parallel zum Anstieg der Gesamtzellzahl. Die Zunahme der Gesamtzellzahl war aber meist stärker als die der Neutrophilen, so dass auch eine Erhöhung der absoluten Zahl der Lymphohistiozyten stattfand, wenn auch die Zellvermehrung vorwiegend auf Kosten der Neutrophilen erfolgte. So war im Fall 8 eine Zunahme der Gesamtzellzahl von 820 auf 10,410 feststellbar, während die Vermehrung der Lymphohistiozyten von 328 auf 728 in cmm ein. Bei 3 Kranken kam es zu einer Abnahme der Neutrophilen, in einem Falle blieb der saumteiwissgehalt schwankte zwischen 5 und 6 % und ahl unverändert.

tion, auch der Blasenbildung, bei Anergie, für die Tuberkulose nicht zutrifft:

Fall 7. H. M. 16j. Mädchen machte 8 Monate vor der Aufnahme eine tbc. Primärinfektion mit Erythema nodosum durch. Beim Eintritt war das volle Bild der generalisierten verkäsigen Tbc des ly. Systems mit grossen Drüsen feststellbar. Der Verlauf war unaufhaltsam progredient, Pat. starb unter den Erscheinungen einer therapierefraktären thrombopenischen Purpura und Peritonitis. Die Sternalpunktion ergab neben einer myeloischen Linksverschiebung eine aplastische Reaktion der Thrombopoiese. Sektion: Schwere verkäsige Tbc der thorakalen, und abdominalen Lymphknoten, der Milz, der Leber, ausgedehnte hämatogene Lungentbc. Die 10 Tage vor dem Tode angelegte Kantharidenblase wies morphologisch 70 % Neutrophile und 30 % Lymphohistiozyten auf, wobei Vakuolisierung und toxische Granulation der Neutrophilen (wie im Blutbild) und die Beteiligung der grossen, gelappt-kernigen Zellen an der ly.-hist. Reaktion auffiel.

In diesem Falle bestand eine Tuberkulinanergie (Mantoux ausgewertet negativ) und auch der klinische Verlauf war anergisch. Die Kantharidenreaktion blieb aber nicht aus, im Gegenteil, wir erhielten eine ziemlich grosse Blase mit 0.9 ccm trübserösem Inhalt, der einen Eiweissgehalt von 6.7 % mit einem Albumin/Globulinverhältnis von 45/55 aufwies, während im Blutserum 7.85 % Eiweiss mit einem Albumin/Globulinverhältnis von 10/90 feststellbar war. Der Befund ist mit der grösseren Permeabilität der Gefässe für die feindispersen Albumine zu erklären, so dass es im Reizexsudat zur Anreicherung der Albumine kam. Das morphologische Bild mit 30 % Lymphohistiozyten sollte eher für eine günstige Prognose sprechen, der Krankheitsverlauf war aber unaufhaltsam progredient und der Tod trat 5½ Wochen nach dem Eintritt in die Heilstätte ein. Die relativ häufigen Versager schränken die diagnostisch-prognostische Bedeutung der KBR erheblich ein und sie ist auch für die Praxis technisch zu zeitraubend. Die Bedeutung der Methode scheint uns auf einem anderen Gebiet zu liegen; sie ermöglicht morphologische Einblicke in die Reagibilität des erweiterten RES, die uns bis jetzt in vivo noch versagt waren.

4. Eiweisschemische Untersuchungen.

Die eiweisschemischen Untersuchungen sind für uns zunächst auch mit Rücksicht auf die Entstehung der Eiweisskörper im RES (Sabin, Heinlein, Rohr, Wuhrmann, Leitner) von Interesse.

6. Andere Untersuchungen.

A. Kongorotversuche. Da die Lymphohistiozyten vom RES abstammen, lagen Untersuchungen über die Veränderungen der Kantharidenblase im akuten Speicherungsversuch nahe. Die Frage, ob das RES im Speicherungsversuch gehemmt oder gereizt wird, kann im akuten Versuch im Sinne der Reizung beantwortet werden. Nach Letterer ist eine völlige Blockade des RES sogar durch langfristige Zufuhr von Schwermetallen (kolloidales Kupfer) nicht zu erreichen, weil immer neue RES-Zellen produziert werden. In eigenen Tierversuchen (Leitner 4) mit Goldverbindungen konnte ebenfalls eine Steigerung der Funktion des RES beobachtet werden. Zur klinischen Prüfung sind verschiedene Methoden mittels Injektion kolloidaler Stoffe angegeben worden. Sie beruhen darauf, dass kolloidale Stoffe, welche in die Blutbahn injiziert werden, im RES abgefangen werden; da ja die Blutgefäße normalerweise nur für echtgelöste Substanzen, nicht aber für Kolloide durchgängig sind, verweilen die grobdispersen Stoffe im Blut, bevor sie im RES abgelagert werden. Wir haben uns der Kongorotmethode von Adler und Reinmann bedient, deren Brauchbarkeit bei Tuberkulose von Alföldy und Bernath, Wedekind, Trautwein u. a. bestätigt wurde. Wedekind benutzte sie zur Unterscheidung zwischen exsudativer und produktiver Tbc, indem er annahm, dass das RES bei produktiver Tbc leistungsfähiger ist und das Kongorot aus dem Blute rascher eliminiert wird, als bei exsudativer Tbc.

Wir wählten die Kongorotmethode, weil sie einfach und unschädlich ist, werden doch intravenöse Kongorotinjektionen zur Behandlung der tbc. Hämoptye empfohlen, weil sie im RES die Abscheidung grobdisperser Eiweisskörper, namentlich des Fibrinogens anregen sollen. Wir haben nach der üblichen Untersuchung des Blaseninhaltes im Vorversuch, 10 ccm 1 %-ige Kongorotlösung intravenös injiziert. Dann haben wir ein neues Kantharidenpflaster aufgelegt und den Blaseninhalt nach 22 Stunden erneut untersucht. Bei 11 derart untersuchten Kranken fanden wir keine verwertbaren Resultate, die positiven und negativen Schwankungen glichen sich aus, so dass wir auf eine tabellarische Wiedergabe der Befunde verzichten.

B. Bellafolinversuche. Um eine eventuelle Abhängigkeit der KBR vom vegetativen Nervensystem zu prüfen, haben wir nach

die Veränderungen nach Tuberkulin waren geringfügig. Immerhin nahm der Eiweissgehalt in 10 von 14 Fällen zu, in je 2 Fällen blieb er gleich bzw. er wurde niedriger. Das Albumin/Globulinverhältnis änderte sich bei 9 Kranken zugunsten der Albumine und bei 4 Kranken zugunsten der Globuline. Die Zunahme des Gesamteiweissgehaltes spricht dafür, dass es infolge der Allergisierung zu einer erhöhten Durchlässigkeit der Gefässe für Eiweisskörper gekommen ist. Die Zunahme der Albumine ist ebenfalls verständlich, weil die Gefässe zuerst für die feindispersen Eiweisskörper durchlässig werden. Die Globulinzunahme war bei 4 Schwerkranken feststellbar, bei 3 mit kavernöser Lungentbc und in einem Falle von doppelseitiger exsudativer Pleuritis. Es ist wahrscheinlich, dass bei Schwerkranken die Permeabilität der Gefässe soweit alteriert sein kann, dass sie dann auch die grobdispersen Globuline durchlassen. Zusammengefasst ergibt sich, dass nach Tuberkulinisierung eine Vermehrung der Gesamtzellzahl und der Neutrophilen, in geringerem Grade auch der Lymphohistiozyten eintrat, ferner eine Vermehrung der Gesamteiweissgehaltes, häufiger zugunsten der Albumine, seltener, namentlich bei Schwerkranken, auf Kosten der Globuline. Die Makrophagenreaktion, die sonst bei allergischen Zuständen beobachtet wurde (Kallós u. a.), war also in der Kantharidenblase nur geringfügig, doch unterscheidet sich letztere von reinen, akut allergischen Gewebsreaktionen.

Die Eiweissbefunde in der Kantharidenblase erklären vielleicht, weshalb in bestimmten Fällen eine exsudative Pleuritis zu fibrinreicher Exsudation mit starker Schwartenbildung führt. Bei schweren, entzündlichen Tuberkulosen kommt es bei einer bestimmten Allergielage zu einer vermehrten Permeabilität der Gefässe und zu Übertritt von grobdispersen Eiweisskörpern (Globuline, Fibrinogen) ins Exsudat (die Verhältnisse im Pleura- und Kantharidenblasenexsudat sind einander ähnlich). Praktisch noch wichtiger ist die Frage, ob es mittels der KBR möglich wäre, die Eigenschaft eines Exsudates im intra- und extrapleuralem Pneumothorax vor der Einleitung der Behandlung zu bestimmen! Es ist bekannt, dass eine stark fibrinöse »plastische« Exsudation zu vorzeitiger Verschwartung des Pnx führt, welche die Wirkung der Kollapstherapie vereitelt. Da die Beurteilung des Fibringehaltes der KBR einfach ist, hätten wir in ihr einen einfachen Test, die vor dem Eingriff (namentlich bei extrapleuralem Pnx) angestellt, wertvolle Hinweise geben könnte.

cantharides blister in clinical practice. This investigation is, however, of undoubted scientific interest because of its relationship to immuno-biological processes and its importance for the knowledge of the reticulo-endothelial system, which play such a large part in the defence mechanism of the body.

Biochemical examinations of cantharides blister fluid showed lower protein values than in serum. Refractometric and viscosimetric methods gave a relative increase of albumen in the blister contents. This can be explained by the greater permeability of vessels to the protein bodies with smaller molecular weight. After the application of tuberculin the total number of cells increased in many cases, mainly at the expense of the polymorphs. The total proteins of the blister contents also increased, mainly at the expense of the albumen fractions. In a few very ill patients the globulin fractions also increased. These observations suggest that the permeability of the vessels increases when allergy sets in. In certain forms of tuberculosis the protein bodies with the greater molecular weight are also let through the vessel walls.

In many cases the blister fluid was rich in fibrin from the start, and it clotted quickly. It is not clear as yet, whether the cantharides blister reaction can make contributions to the solution of other problems, why, for instance, in certain cases of pleurisy the exudate which is rich in fibrin leads to thick plaques, the so-called »plastic pleurisy». Further it is still unknown, why extrapleural pneumolysis in extrapleural pneumothorax produces an exudate rich in fibrin, which can rapidly lead to diminution or even obliteration of the pneumothorax. The success of an operation is often endangered by this phenomenon. Investigations will be welcome, to ascertain if the cantharides blister reaction carried out before operation can provide guidance as to whether or not the development of an exudate is to be expected. The estimation of the fibrin in the contents of an exudate, we would have a simple test which would be of great practical importance in deciding on operation.

Literaturverzeichnis.

1. Adler, H. und Reimann, F.: Z. exper. Med. 47, 617 (1925). — 2. Alder, A. und Zaruski, M.: s. Dissertation M. Zaruski. Über Eiweissveränderungen von Blutserum und Gewebeflüssigkeit bei Lungentbc. Zürich 1926. —

From the Department of Obstetrics and Gynaecology of the
University of Amsterdam.

(Chief: Prof. M. A. van Bouwdijk Bastiaanse.)

Hyperglobulinemia and Pregnancy.

By

G. A. LINDEBOOM.¹

M. D.

(Submitted for publication November 3, 1947.)

Introduction. Already in the earlier decades of this century great attention by various authors was directed to the protein content of the serum of pregnant women. A diminished level seemed to be connected with the hydremia and the tendency to edemaformation in pregnancy.

However, the methods employed for the determination of the protein are known to yield not invariably reliable results.

Later the different protein fractions were studied by various authors. Generally they agreed that the decrease of protein was caused by a lowering of the albumin fraction.

Plass and Matthew (1) reviewed the literature up to 1926, and made an important contribution. Using the colorimetric method of Wu, they analysed 314 plasmas of non-pregnant, pregnant, parturient and puerperal women. They concluded that the serum-albumin is diminished to such an extent that the fall in the total protein may be attributed to the reduction in the concentration of this fraction. In the last lunar month an average of 4.07 % albumin was noted.

Recently Rinehart (2), using the colorimetric biuret method of Robinson and Hogden, published the results of 251 determinations of 79 normal pregnant women and 39 determinations on 5 pre-eclamptic women.

¹ Frans van Mierisstr. 41, Amsterdam.

Table I.

Serum Protein Content in Normal Pregnancy with Sufficient and Insufficient Protein Supply, in Toxemic Pregnancy and in Eclampsia.

Women in the last trimester of pregnancy	Total protein g %		Albumin g %		Globulin g %	
	Range	Average	Range	Average	Range	Average
24 normal women with protein supply of 60-100 gram	5.96-7.07	6.38	3.63-4.69	3.97	1.72-3.05	2.41
36 normal women with protein supply less than 60 gram	5.36-7.2	6.29	2.85-4.44	3.94	1.62-3.16	2.35
These 60 normal women	5.36-7.2	6.32	2.85-4.69	3.95	1.62-3.16	2.38
31 Women with toxemia	4.35-7.47	6.05	2.23-4.28	3.58	1.41-3.53	2.48
33 Women with eclampsia	4.58-8.33	6.10	2.78-4.63	3.61	1.70-3.70	2.48

In normal pregnancy he found a decrease in the albumin content from 4.7 to 4.0 % in the eighth and ninth months. According to Strausz (3), whose important work drew much attention, the hypalbuminemia of pregnancy is due wholly, or partly, to an insufficient intake of protein. This hypalbuminemia was more pronounced in toxemia, and most in eclampsia. Strausz found for normal pregnant women with an average, and those with a poor, protein diet, for patients with toxemia and eclampsia respectively, a mean albumin value of 3.7, 3.5, 3.1 and 2.6 %. Also Dodge and Frost (4) noted a certain correlation between protein intake and the albumin-level of the serum in pregnant women.

We ourselves have failed to find any significant difference between the albumin content of the serum in women with a normal and a low protein diet. In 24 women with a daily protein supply of 60-100 g, we found an average albumin content of 3.97 %, in 36 women with insufficient protein-intake 3.94 %, in 31 women with non-convulsive toxemia 3.58 %, and in 33 eclampticae 3.61 %. (Table I.) Several women with eclampsia showed an entirely normal level of total protein and albumin. In this regard our results do not agree with the statements of Strausz.

The Globulin-level in Pregnancy.

Undoubtedly the most marked changes of the serum protein concern the albumin fraction. Less attention was paid to the globulin content, though Eufinger (5), working with the refractometric method, as early as 1928, noted a tendency to rise.

Plass and Matthew found a slight relative increase, although the absolute values remained practically normal. They noted a rise of globulin on the third day of puerperium, which they thought associated with the onset of milksecretion. They noted a great individual variation and often found values above 3 %. The range and average in normal non-pregnant women (2.32—3.07 %, average 2.69 %), however, is higher than reported by other authors. Rinehart, who found in normal non-pregnant women a globulin content of 1.99 % (1.5—2.4 %), obtained for the pregnant women an average value of 2.3 %. This mean value remained almost constant, in the different stages of pregnancy though it was at all times slightly higher than that for the non-pregnant state. His table shows that in the last 4 months of pregnancy values of 3 % and higher were found. Only one of the five cases with pre-eclampsia showed in two out of ten determinations a globulin-level above 3 %.

Dodge and Frost found for globulin in the first trimester of pregnancy a mean value of 1.5 %, in the second trimester 2.11 %, and in the third trimester 1.99 %. For the patients with toxemia they found a mean value of 2.07 %.

They considered this slight rise in the globulin as a new observation which should be verified.

Strausz, in his study of 65 pregnant women, found no correlation whatsoever between diet, toxemia or eclampsia, and the level of globulin. According to the chart the mean level lay between 2.3 and 2.4 %.

Our own Investigations.

Laboratory procedure: For the protein determinations we used the method of Howe — Torsten Teorell. This method had been shown to be reliable by various authors (Kruysveldt (6), de Vries (7)). In normal non-pregnant women we found for the albumin fraction values of 4.4—5.3 % (average 4.9 %), and for the glob-

Table II.

46 Cases of Hyperglobulinemia During Pregnancy.

Number	Days before delivery	Total protein g %	Al- bumin g %	Glo- bulin g %	Clinical diagnosis	RR	Alb. % ₁₀₀	Remarks
50—P 51	?	7.07	4.02	3.05	N			
55—13943	4	6.33	3.29	3.04	T	145/105	3	
57—13930	17	7.81	4.28	3.53	T	185/120	1/4	
71—14073	7	6.39	3.04	3.35	T	170/130	3	
72—14074	2	6.24	3.18	3.06	T	180/110	8	pyelitis p. p.
90—14184	25	6.87	3.61	3.26	T	165/125	6	
94—14345	29	7.10	3.94	3.16	N			
95—14246	10	6.98	3.90	3.08	N			
112—14355	22	7.26	4.14	3.12	T	185/120	1/4	
128—14460	31	7.72	4.14	3.58	T	185/125	1/2	
147—14573	0	7.19	3.72	3.47	E (40 conv.)	145/110	12	
169—13952	25	8.22	3.37	4.85	P	110/80		
171—14685	2	7.21	4.23	3.01	P—E	200/120	trace	Child †
191—13296	24	7.14	3.80	3.34	T	153/90	3	
193—14928	3	6.63	3.36	3.27	P			
207—15304	1	8.57	4.53	4.04	PE	240/170	1/2	
246—13195	14	7.79	4.61	3.18	T	175/130	1/2	
252—15708	137	7.44	4.30	3.14	H—T	200/130	1/2	
272—12660	14	7.29	4.26	3.03	T	170/120	1/4	
312—15930	5	6.67	3.57	3.10	P	130/75	3/8	
313—15933	3	6.88	3.74	3.14	N			
325—15960	0	7.02	3.37	3.65	PE	190/100	7 1/2	
350—16041	55	7.91	3.65	4.26	P	155/100	1/2	
380—16151	63	7.77	4.22	3.55	P	120/90	+	
390— 9895	6	5.78	2.66	3.12	T	165/110	3	
402—13525	1	7.55	4.44	3.11	N			
410—16302	1	6.83	3.80	3.03	H	255/165		U+ 900 mg/L
420—16299	14	6.20	3.18	3.02	N (slight pyelitis)	125/90		
425—PV	?	8.58	5.33	3.25	N			
429—16446	2	8.16	4.83	3.33	N (append. ac.)			
438—16569	34	8.33	4.63	3.70	E (super- poned on chron. neph.)	220/140	3	died after 1/2 year
461—15086	7	7.90	3.95	3.95	N lues cong.	120/85		
463— 9699	1	7.61	4.29	3.32	T	155/80	trace	
466— 1972	43	7.47	4.03	3.44	H	200/120	+	
473—16782	7	7.52	4.49	3.03	T	165/115	3/4	
475—15415	21	8.13	4.90	3.23	H	160/120	trace	prev. pregn. E
477—16795	2	7.24	4.13	3.11	T	150/95	6	
480—10213	3	7.46	4.38	3.08	T	155/105	trace	
485—16814	169	7.82	4.63	3.19	Haematuria e causa ignota			
488—16827	19	7.07	3.94	3.13	H—PE	240/150	3	
500—16992	36	7.00	3.80	3.20	N			
504—16983	2	6.93	3.82	3.11	E	140/100	1 1/2	
508—16779	73	6.82	3.61	3.21	Neph. chron.	190/135	+	
510—12783	1	8.56	4.52	4.04	E			died undeliv- ered
537—17679	1	7.27	4.14	3.12	T	160/120	9	
540—14080	32	5.98	2.62	3.36	T	135/95	+	

N normal pregnancy
T toxicosis
E eclampsia

P pyelitis gravidarum
PE prae-eclampsia
H (prae-exsistent) hypertension

Table III.

15 Cases of Hyperglobulinemia in the Puerperium.

Number	Days before delivery	Total protein g %	Al- bumin g %	Glo- bulin g %	Clinical diagnosis	RR	Alb. %	Remarks
239-15643	1	6.88	3.65	3.23	H	160/100	trace	prev. pregn.E
264-15714	1	6.77	3.62	3.15	E lues latens	180/120	28	WR —
289-15725	46	7.58	4.48	3.10	T	190/150	9	
304-15872	9	7.09	3.70	3.39	E (intercur.)	220/100	3½	
309-1624	9	8.04	4.56	3.48	T	180/130	+	
337-14766	9	6.77	3.68	3.09	T	185/125	½	prev. pregn.E
385-15957	9	7.28	4.28	3.00	P	120/80	trace	
392-15752	2	5.61	2.51	3.10	Chron. neph.	190/130	1½	
444-16621	1	7.32	4.17	3.15	T?	150/105	trace	
498-2510	9	7.19	4.16	3.03	T	200/130	1	prev. pregn.
506-16629	9	7.56	4.56	3.00	H	170/100	trace	PE
512-17099	1	7.09	3.94	3.15	H	190/115	+	
520-17156	10	7.59	3.84	3.75	T	180/100	3½	
529-17293	1	6.61	3.43	3.18	H+E(interc.)	240/130	6½	
535-16484		6.50	3.30	3.20	Hydrops foet.	110/75	+	

ulin 1.8—2.4 % (average 2.2 %). In the preliminary series of protein determinations, summarized in table I, our attention was drawn to the fact that a globulin content of 3 % or higher occurred twice in the 60 normal pregnant women, but 6 times in the 31 cases of toxemia. Of the 33 cases of eclampsia four had a globulin level of 3 % or higher. This preliminary research suggested a higher incidence of a high globulin level in toxemia.

Once our attention had been called to the fact of the more frequent occurrence of hyperglobulinemia in toxemia than in normal pregnancy, we decided to search our records on this phenomenon. In calculating the averages, the extreme values disappear to a certain extent. Therefore it seemed desirable to see how many times a high globulin level was noted.

There is no general opinion as to the upper limit of the normal globulin content; some put this at 2.4, others at 3 %. (Bing (8)). When, in this paper, we speak of hyperglobulinemia, we mean a globulin level of at least 3 %.

Incidence: From 1941 till 1944 (when war conditions necessitated a cessation in our investigations) 491 protein determinations were made in 295 women before or after delivery. More than two-thirds of these women suffered from some toxemia or other complication of pregnancy. Most, but not all of the women who were examined after delivery, had also been examined earlier during pregnancy. In 46 cases hyperglobulinemia was noted

ALBUMIN CONTENT
IN 46 CASES OF HYPERGLOBULINEMIA DURING PREGNANCY
AND 15 CASES AFTER DELIVERY

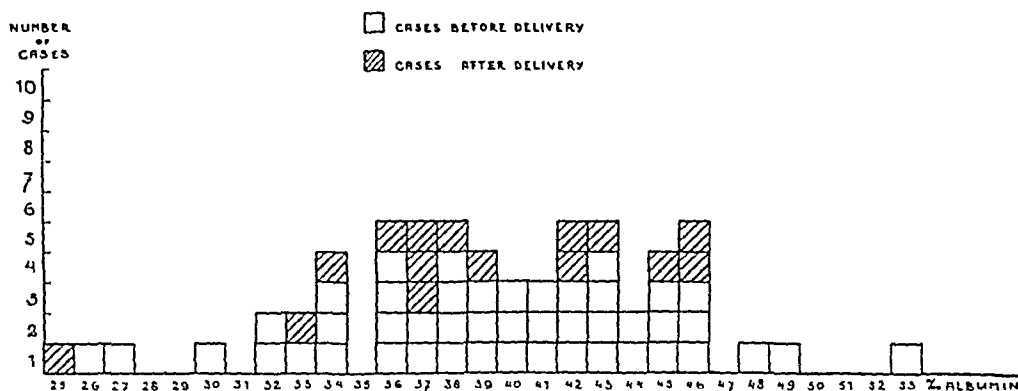


Chart 1. The albumin content (in ‰) in the cases of hyperglobulinemia.

during pregnancy, in 15 cases during the puerperium, therefore 61, *i. e.* 20.8 % of the 295 women examined showed an undeniable hyperglobulinemia at some time of pregnancy or puerperium. Of the 46 pregnant women with hyperglobulinemia 10 had a globulin content of the serum of 3.5 % or higher; of the 15 puerperae 1 (see table II and III).

Relation to albumin. As the rise of the globulin might be a compensatory mechanism for the hypalbuminemia, a consideration of the albumin values is indicated. As, according to our experience, during pregnancies the normal value for albumin is 3.95 %, a glance at chart I shows that the albumin level lies almost as many times above this level as beneath it. Therefore the hyperglobulinemia cannot be considered simply as a compensatory effect.

Course. It seems interesting to know what happens in these cases after delivery. As a rule, when determined repeatedly, the globulin content shows marked variations in the pregnant women. Of the 61 women 27 were examined twice or more oftener in the first weeks after delivery. Twelve times the globulin content fell beneath 3 %, 15 times it remained above 3 % (4 times at 3.5 % or higher).

The women who showed a hyperglobulinemia at the last examination after delivery, were summoned in 1947 (3—5 years later) for a follow-up examination; 12 women could be examined (table V).

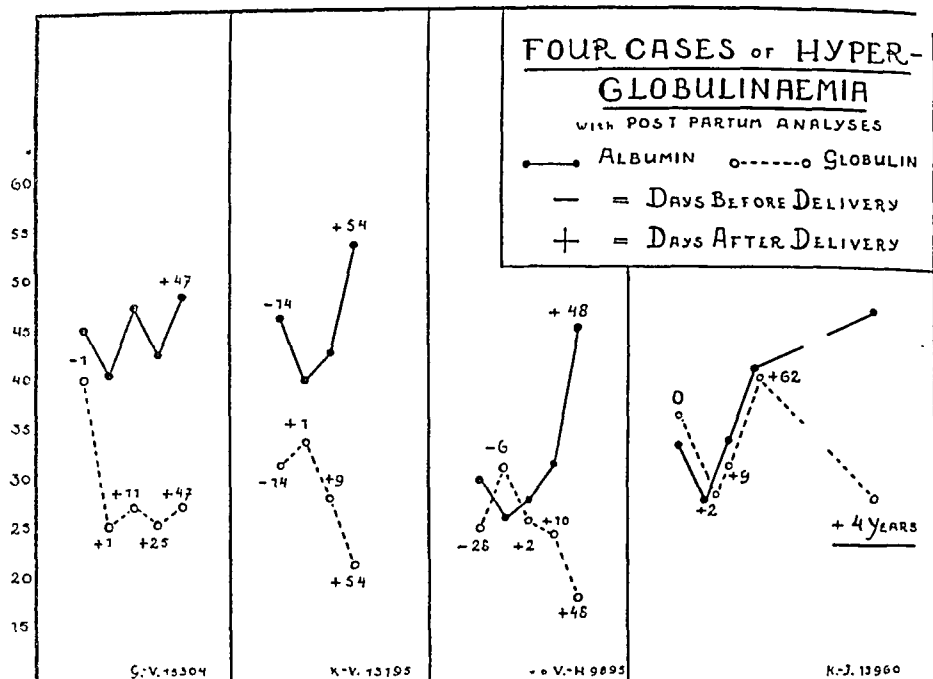


Chart. 2. Serum protein determinations in 4 cases with hyperglobulinemia before and after delivery.

Table IV.

Hyperglobulinemia ($>3\%$) and Pregnancy.

491 determinations on 295 women (more as $\frac{2}{3}$ suffered from toxemia)

I a. before delivery 46 cases (10 with 3.5 % or more)

b. after " 15 " (1 " 3.5 % " ")

Total 61 cases (20.8 % of 295 women).

II of these 61 women 27 were examined twice or more times shortly after delivery.

Result: fall beneath 3 %: 12 cases
 remaining above 3 %: 15 "
 (" at 3.5 % or higher: 4 ").

III 12 women with hyperglobulinemia at the last examination after delivery were re-examined after 3-5 years.

Result: 3 cases with persisting
 Hyperglobulinemia (3.33, 3.65 % and 3.09 %).

In this series three times hyperglobulinemia appeared to be persistent. Therefore in women with hyperglobulinemia of unknown origin, the possibility should be borne in mind that the

Table V.

Control Examination after 3—5 Years of 12 Cases with Hyperglobulinemia at the Last Examination after Delivery.

Number	Days (years) a. p. or p. p.	Tot. prot. g %	Albumin g %	Globulin g %
16151.....	63 a. p.	7.77	4.22	3.55
	1 p. p.	5.71	2.99	2.72
	9 p. p.	7.88	4.40	3.48
	5 years	7.11	5.08	2.03
15960.....	0 a. p.	7.02	3.37	3.65
	2 p. p.	5.72	2.84	2.88
	9 p. p.	6.55	3.40	3.15
	62 p. p.	8.13	4.13	4.00
	5 years	8.03	4.70	3.33
17682.....	8 a. p.	7.52	4.49	3.03
	1 p. p.	7.94	4.18	3.76
	10 p. p.	7.24	4.05	3.19
	4 years	5.58	5.07	2.51
10213.....	4 a. p.	7.46	4.38	3.08
	1 p. p.	6.92	4.08	2.84
	9 p. p.	7.10	4.20	2.90
	4 years	7.76	4.87	2.89
16983.....	2 a. p.	6.93	3.82	3.11
	1 p. p.	5.25	3.12	2.13
	9 p. p.	7.79	4.45	3.34
	4 years	7.13	5.23	1.90
15872.....	2 a. p.	5.62	2.99	2.63
	1 p. p.	4.74	2.59	2.15
	9 p. p.	7.09	3.70	3.39
	58 p. p.	8.38	3.89	4.48
	5 years	7.75	4.10	3.65
16629.....	78 a. p.	6.94	4.21	2.73
	1 p. p.	6.62	3.81	2.81
	9 p. p.	7.56	4.56	3.00
	127 p. p.	9.19	6.04	3.15
	4 years	7.29	5.12	2.17
15752.....	190 a. p.	6.17	3.99	2.18
	2 a. p.	5.74	3.04	2.70
	2 p. p.	5.61	2.51	3.10
	9 p. p.	5.72	2.60	3.12
	5 years	6.49	4.14	2.35
17293.....	28 a. p.	6.63	3.93	2.20
	1 p. p.	6.61	3.43	3.18
	7 p. p.	7.12	7.02	3.10
	48 p. p.	8.45	5.25	3.20
	3½ year	7.43	5.04	2.39
15086.....	7 a. p.	7.90	3.95	3.95
	2 p. p.	6.53	3.48	3.05
	10 p. p.	7.07	3.91	3.16
	4½ year	7.51	5.10	2.41
16484.....	23 a. p.	5.84	3.11	2.73
	1 p. p.	6.50	3.30	3.20
	8 p. p.	8.25	4.74	3.51
	3½ years	7.65	4.82	2.83
13952.....	25 a. p.	8.17	3.37	4.80
	273 p. p.	8.27	4.61	3.66
	4½ years	7.88	4.79	3.09

high globulin level originates from a previous pregnancy. In one of these cases the globulin content rose from 2.61 % ante partum to 3.39 % in the puerperium; five years later it was 3.65 %. However, as long as protein determinations before pregnancy are lacking, there remains in these cases the possibility of a pre-existing hyperglobulinemia of unknown origin.

Pyelitis of Pregnancy.

In our records 6 cases of pyelitis of pregnancy showed a hyperglobulinemia (see table VI). Some of these patients evinced a transient icterus or hyperbilirubinemia. The globulin content was sometimes very high. Hyperglobulinemia seems a fairly common symptom in the more severe cases of pyelitis of pregnancy. This hyperglobulinemia is not always persistent.

Table VI.

6 Cases of Pyelitis of Pregnancy with Hyperglobulinemia.

Number	Days a. p.	Proteins (g %)		
		Total	Albumin	Globulin
169-13952.....	25	8.22	3.37	4.85
193-14928.....	3	6.63	3.36	¹ 3.27
312-15930.....	5	6.67	3.57	3.20
350-16041.....	55	7.91	3.65	¹ 4.26
380-16151.....	63	7.77	4.22	¹ 3.55
-21889.....	15	7.18	3.85	3.33
Average		7.10	3.67	3.73

¹ Afterwards normal globulin level.

The Globulin Fractions.

In 10 cases of hyperglobulinemia during pregnancy, or puerperium, a determination of the fractions of the globulin (euglobulin, pseudo-globulin I and pseudo-globulin II) were carried out. We accepted the normal standards, as worked out by De Vries (7), who used the same method as ourselves. In the recent literature we found no determinations of the globulin fractions in pregnant women. In a small series of 4 pregnant women with a normal globulin content we ascertained that our values agreed essentially with these of De Vries. (Table VI). (The globulin

Table VII.

The Fractions of Globulin in 4 Pregnant Women with a Normal Globulin Content.

Number	Total Protein g %	Albumin g %	Globulin (by subtraction) (fractions added) g % g %		Pseudo-Globulin II g %	Pseudo-Globulin I g %	Eu-Globulin g %
76—327	6.60	3.93	2.67	2.79	1.02	1.12	0.65
77—89	6.38	4.09	2.29	2.38	0.80	1.15	0.43
86—14164	5.70	3.57	2.13	2.22	0.74	0.91	0.57
87—609	6.11	4.01	2.10	2.18	0.71	1.08	0.39
Average.. Normal range..	6.20	3.90	2.30 (1.64-2.51)	2.39 (1.70-2.63)	0.82 (0.34-0.65)	1.07 (0.95-1.52)	0.51 (0.21-0.58)

Remark. For the globulin two values are given; the first is found by the subtraction of the albumin N and the rest N from the total N, and then multiplying by 6.3. The second value is obtained by addition of the different fractions, for which the multiplication factors, according to a slightly different N content, deviate a little from 6.3.

Table VIII.

The Fractions of Globulin in 10 Sera (7 Pregnant Women, 3 Puerperae) with a High Globulin Content. (See Remarks at Table VII.)

Number	Total Protein g %	Albumin g %	Globulin by subtraction (fractions added) g % g %		Pseudo-globulin II g %	Pseudo-globulin I g %	Eu-globulin g %
98—14184	6.64	3.21	3.43	3.56	0.77	1.37	1.42
106—P 457	7.10	3.66	3.44	3.51	0.66	1.12	1.73
113—14355	6.89	3.94	2.95	3.07	0.94	1.36	0.77
134—14460	7.77	4.11	3.66	3.76	0.88	1.53	1.35
154—13930 ¹	7.90	3.56	4.34	4.56	2.13	1.52	0.91
156—14573	7.05	4.03	3.02	3.13	0.80	1.65	0.68
169—13952 ¹	8.27	4.61	3.66	3.76	0.83	1.82	1.11
172—14685 ¹	7.17	4.17	3.00	3.08	0.68	1.69	0.71
193—14928	6.63	3.36	3.27	3.37	0.71	2.00	0.66
540—14080	5.98	2.62	3.36	3.50	1.34	1.06	1.10
Normal range ...			1.64-2.51	1.70-2.63	0.34-0.75	0.95-1.52	0.21-0.58

¹ Post partum.

fractions were precipitated in Na_2SO_4 concentration of 21.3, 17.4 and 13.5 %.)

Table VII shows that in the cases of hyperglobulinemia the high globulin level is due to an increase in different fractions. The

eu-globulin content was always higher, the pseudo-globulin II content was 4 times slightly, and twice markedly, increased, and the pseudo-globulin I showed 4 times a moderate to marked rise.

Conclusions.

1. In the last trimester of normal pregnancy there is a definite tendency to a rise of the globulin level of the serum; in some cases a hyperglobulinemia is found (3 % and higher).

2. In cases of non-convulsive toxemia the percentage of hyperglobulinemia is much higher (about 20 %).

3. In a series of 33 cases of eclampsia four times hyperglobulinemia was noted.

4. In 10 cases of hyperglobulinemia in pregnancy or puerperium a determination of the eu-globulin, pseudo-globulin I and pseudo-globulin II was carried out; there was in every case a rise of the eu-globulin level.

5. In a series of 60 cases (not further analysed here (9)) the protein fractions of the serum were determined near the time of delivery and twice or more in the puerperium; we got the impression that shortly before delivery hyperglobulinemia is more frequent than in the earlier weeks of the third trimester; in several cases hyperglobulinemia occurred for the first time after delivery.

In the cases of hyperglobulinemia before delivery often a fall was noted on the second day after delivery.

6. In pyelitis of pregnancy hyperglobulinemia is often found.

7. Twelve women with a hyperglobulinemia, present at the last examination (2—3 months) after delivery, were re-examined 3—5 years later; in 3 cases the hyperglobulinemia apparently persisted.

8. These results seem to suggest a disturbance in the protein metabolism during toxemia of pregnancy.

Summary.

In this paper the occurrence of hyperglobulinemia during the last trimester of pregnancy and in the puerperium is considered.

Literature.

- 1) Plass and Matthew. *Am. J. Ob. Gyn.* 12, 346, 1926. — 2) Rinehart. Serum protein in normal and toxemic pregnancy. *A. J. Ob. Gyn.* 50, 48, 1945. — 3) Strausz. Observations on the etiology of the toxemias of pregnancy. *Am. J. Med. Sci.* 190, 811, 1935 (I), 195, 723, 1938 (IV). — Strausz. Toxemia of pregnancy. *Am. J. Ob. Gyn.* 38, 199, 1939. — 4) Dodge and Frost. Relation between blood-plasma proteins and toxemia of pregnancy *J. A. M. A.* 11, 1898, 1938. — 5) Eufinger, *Klin. Woch.* 7, 492, 1928. — 6) Kruysveldt, *Onderzoek over de bloedeiwitten etc.* Dissertation, Amsterdam, 1936. — 7) De Vries. *Over de reactie van Takata-Jezler etc.* Diss. Amsterdam, 1938; See also *Acta med. scand.* 99, 425, 1939. — 8) Bing. Hyperglobulinemia, *Acta med. scand.* 103, 547, 1940. — 9) Lindeboom. *Schweiz. med. Woch.* 76, 461, 1946.
-

Pelger-Huët's Anomaly of the Nuclei of the Leucocytes.

By

ERIC JONSSON, LISA BOSTRÖM and BIRGER BRINGEL.

(Submitted for publication November 10, 1947.)

In view of the fact of the first cases, as far as we know, in Scandinavia of Pelger's familiar anomaly of the leucocytes (»false shift to the left») having been observed at Södersjukhuset (the South Stockholm Hospital), some of them in the past year, publication in this journal would seem to be warranted.

This anomaly received its name from the Dutch hematologist Pelger, who in 1928 gave an account of two cases with the characteristic blood picture described below. His countryman, the pediatricist Huët, determined the hereditary nature of the anomaly.

Pelger-Huët's anomaly would seem to be prevalent in various races and numerous cases (see survey in Nachtsheim) have been described from Holland, Germany and Switzerland, where the matter has been given a special interest, particularly by Undritz in Switzerland.

The heredity of Pelger-Huët's anomaly is dominant and is not confined to sex. In two of the cases published by Stahel and Zündel, however, the customary heredity course could not be demonstrated (the same applies to our case No. 2). We shall return to this question.

Only heterozygotes have so far been ascertained in human beings.

The feature which strikes one above all in examination of blood from a Pelger individual (fig. 1 and 2) is the seemingly pronounced shift to the left. It is not unusual for figures of up to 50 % unsegmented leucocytes to be found.

Whereas normal immature cells have a relatively bright and loose nucleus, the Pelger cells have a dark coarsely pitted nucleus that is rather small in relation to the cell generally.

The segmented Pelger cells hardly ever have more than two segments.

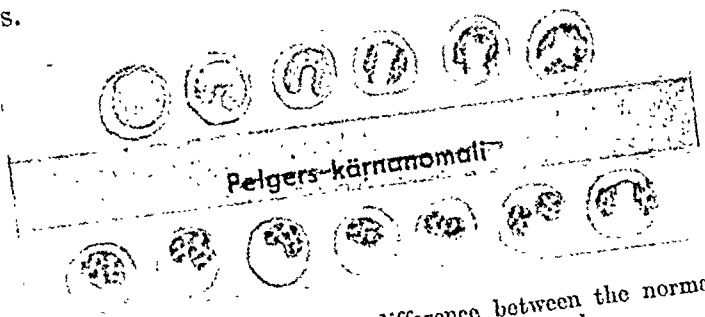


Fig. 1. Skeleton drawing showing the difference between the normal nucleus formation and Pelger-Huët's anomaly.

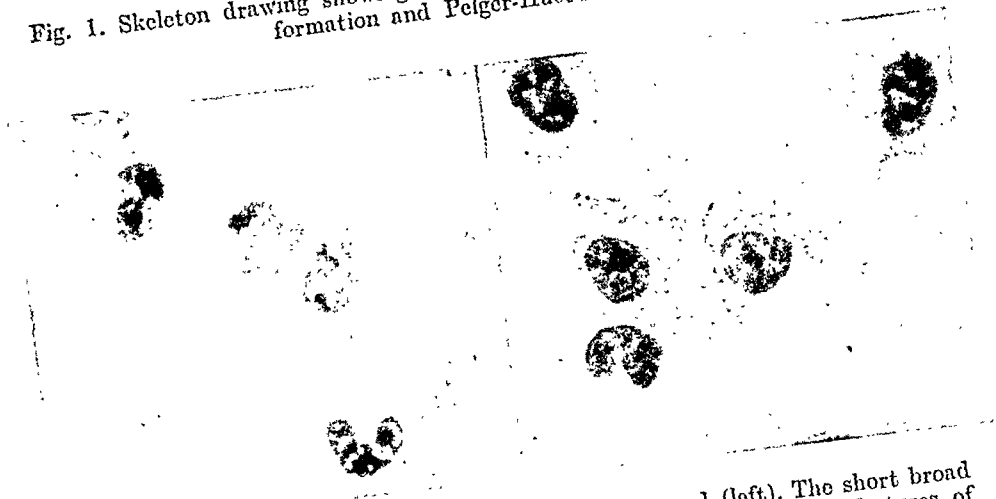


Fig. 2. (Micro-photo 1,000 \times). Blood smear from case 1 (left). The short broad nuclei and the coarse pillared nuclear structure are characteristic features of Pelger-Huët's anomaly. Case 4 (right). Blood picture strongly affected toxically. Some of the nuclei round. Toxic granulation.

The segmentation repression is associated with the chromatin in the Pelger nuclei being highly coherent (Undritz). Colloid chemistry factors contribute (Schmidt).

The characteristic Pelger structure may be found already in the half mature myelocytes. Sternal puncture (fig. 3) moreover shows increase in the number of semi-mature and mature myelocytes and also metamyelocytes. On the other hand the rod and segment nuclear leucocytes are decreased.

Pelger-Huët's anomaly is irreversible.

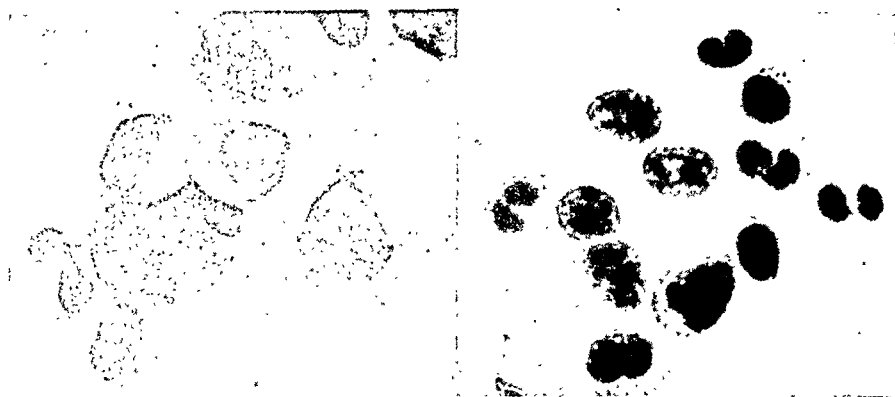


Fig. 3. (Micro-photo 1,000 \times .) Sternal puncture from case 1 (left) and case 3 (right). All stages of development up to segment nuclear are represented. The nucleus has never more than 2 segments.

Undritz calls the ordinary Pelger cases »Voll-Träger» (full-carriers), that is, Pelger-Huët's anomaly occurring in all the leucocytes of the person concerned. Kokubo's »atypical Pelger-Huët's anomaly» — also designated »semi-Pelger» — is characterised by rather stronger tendency to segmenting. Undritz proposed that such cases should be called »Vollträger with mainly bisegmented neutrophile nuclei». These cases also are bearers of Pelger gen. This, however, is not the case with the cases designated »Teilträger» (partial carriers), in which both normal and Pelger leucocytes occur. Usually it is a question of partial carriers, occurring in Pelger families and having less than 1 % of Pelger leucocytes; in 1 case Undritz found an abundance of Pelger cells (»Poly-Teilträger»). Leitner has described a special type of partial carriers.

In the clinical respect Pelger-Huët's anomaly is of comparatively great significance. According to Undritz, patients with Pelger-Huët's anomaly, with the incorrectly interpreted blood picture as indication, have been treated for the presence of tuberculosis, in other cases they have been subjected to appendectomy. In other cases again prolonged and comprehensive examinations have been made unnecessarily, as in our case No. 1.

Whether Pelger-Huët's anomaly in itself has any clinical significance has been the subject of conflicting opinions. It has not been possible to demonstrate with certainty any functional inferiority in the Pelger cells.

Many authors consider the heterozygotic Pelger persons quite

as healthy as persons with normal leucocyte nuclei, whereas others have thought they could show a diminished resistance to infections, including tuberculosis, and accumulated existence of various other disease conditions, such as allergic diseases (Leitner and Leeuwen), hyperthyroidism (Peterson), psychic diseases (Lorenz) etc.

Three of our cases were admitted to the hospital on account of infectious condition.

Nachtsheim does not consider that Pelger-Huët's anomaly may be regarded as a variety of no significance, but thinks it is possible for it to have pathological significance. He supports his opinion on the extremely interesting experiments carried out by him on the initiative of Undritz.

Undritz had demonstrated that Pelger-Huët's anomaly also occurred in rabbits. (Pelger cells occur both normally and as anomalies in various animals (see Schmidt and Undritz, among others).) By pairing heterozygotic Pelger rabbits, Nachtsheim was successful in obtaining homozygotic rabbits («super-Pelger»). The blood picture of these animals was particularly noteworthy. All the leucocyte nuclei were perfectly round and the nuclear structure extremely coarse, so that the chromatin formed large clumps. The majority of the homozygotic Pelger rabbits died before birth and those that survived displayed dwarf growth and various grave malformations, in the extremities and other places.

It would seem, therefore, as if the Pelger gen were not so harmless from the point of view of race hygiene. For the human being, a more complicated organism than the rabbit, it has perhaps a still more deleterious effect in double dose than with that animal (Undritz, Nachtsheim). In any event it should be of interest to investigate the leucocytes in human beings having extremity and other malformations such as agree with those in the homozygotic rabbits.

According to Undritz these questions are of great importance for comparative hematology, in so far as the observations made suggest that the leucocytes in mammals and other vertebrates as well as invertebrates are of the same nature.

At Södersjukhuset, Stockholm, the following 4 cases¹ of Pelger-Huët's anomaly of the nuclei of the leucocytes have been observed:

¹ We wish to convey our thanks to the senior physicians, Docent Ernst Sahlgren and Docent Ernst B. Salén, who so kindly allowed us to have at our disposal cases 2 and 4 respectively, as also to Docent Nils G. Nordensson, who checked the sternal punctures of our cases.

Case 1. T. L. Journal No. 1763/45. *Diagnosis:* Rheumatic fever? Pelger-Huët's anomaly of the nuclei of the leucocytes.

25 years old assistant nurse, sent in for investigation of pains in the joints, subfebrility and rise in sedimentation rate.

Heredity: Great-grandfather Finnish citizen, died at the age of 87. Grandfather, 82 years old, healthy in the main. Grandmother cripple following articular rheumatism (?). The mother has had tuberculosis of the lungs.

Social-hygienic circumstances: Two other children of same parents healthy. Nullipara. Menses, no remarks. Venerea denied.

Previous illnesses: Erythema nodosum as a child and gland on the lung. Otherwise mainly healthy.

Present illness: Throughout the spring of 1945 troubled by sore throat. Colpitis symptoms in the period March—May. At the beginning of April trauma in left knee. Two to three weeks later ache in the knee and stiffness in the mornings. Palpation tenderness over the lateral tibia condyle and over the heads of the gastrocnemius. Objectively nothing definite. S. R. 40 mm/h. Subsequently trouble as above with the knee and also with the left ankle. Nothing objective. Patient given sick leave owing to rising S. R., up to 107 mm/h. but while confined to bed had trouble also with right knee and ankle, and was therefore admitted to Södersjukhuset.

Status showed good general condition, few objective symptoms in the joints and nothing of interest in the internal organs.

Blood: S. R. 83 mm/h. Increase of the globulins. Hb 79 %. Red blood cells 3.79 million. Index 1.03. Anisocytosis and polychromasia. White blood cells 5,700.

Differential count: Metamyelocytes 12 %, rod nuclear 32 %, segment nuclear 15 %, basophile 0 %, eosinophile 3 %, lymphocytes 30 %, monocytes 8 %. (Fig. 1.)

Sternal puncture: Specimen abundant in cells. In the myelopoiesis, shift to the left. Erythropoiesis no remark. Megakaryocytes numerous. Reticule strongly hyperplastic with increase of plasma cells.

Diagnosis: Chronic irritation.

8/8: Still pronounced shift to the left and normal total number white blood cells. Such strong increase of plasma cells in the sternal puncture as to cause suspicion of myeloma. Cranium X-ray negative. From what could be judged it appeared that a serious disease condition was present. The patient was given sulfa and penicillin treatment. During time in hospital regress of the trouble and falling S. R. The whole time continuing shift to the left, the nature of which was uncertain until 25/9, when it was ascertained that Pelger-Huët's anomaly of the nuclei of the leucocytes was present.

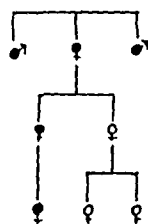
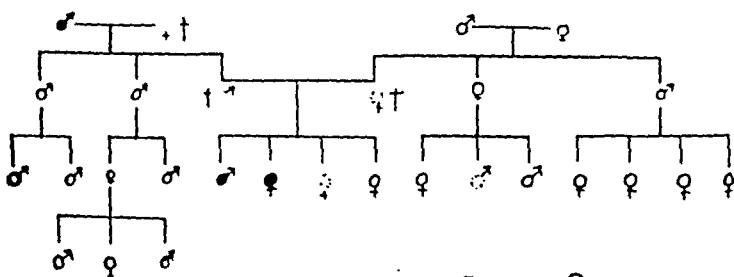
25/9: *Diff.:* metamyelocytes 15 %, rod nuclear 39 %, segment nuclear 11 %, eosinophile 5 %, basophile 0 %, lymphocytes 25 %, monocytes 5 %, plasma cells 0 %.

Nuclei of the neutrophile cells small and coarsely pitted. Never more than two segments.

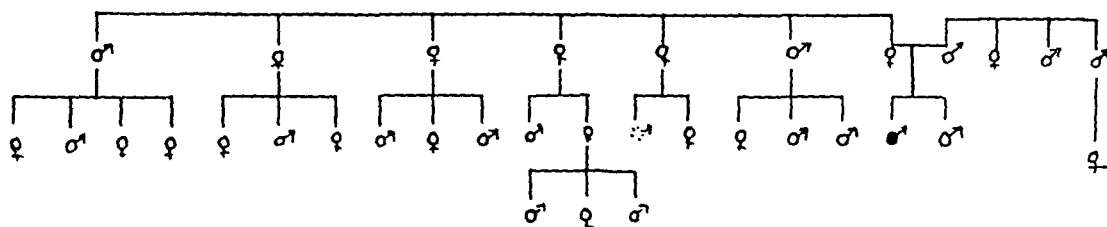
On investigation of family (fig. 4), Pelger-Huët's anomaly could

Case 1

Case 4



Case 2



Case 3

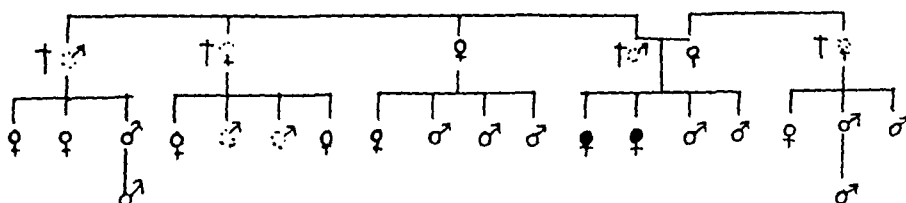


Fig. 4. Genealogical trees. Shaded circles = Pelger cases. Unshaded circles = normal cases investigated. Hatched circles = persons not investigated.

It was the father of case 1 who must have transmitted the Pelger anomaly from the paternal grandfather (see genealogical tree). In case 3 likewise the father would seem to have transmitted the Pelger trait. — In case 4, as in case 1, the Pelger anomaly is found in 3 generations. In this instance, however, it could also be demonstrated in the intermediate generation. — It should be observed that a man of family 1 is married to a woman of family 2. Both these have normal leucocytes; this obviously is also the case with their children.

be ascertained in the paternal grandfather and a brother. Parents dead.

Re-examination one year later:

17/6—46: *Diff.*: Myelocytes 1 %, metamyelocytes 3 %, rod nuclear 46 %, segment nuclear 11.4 %, eosinophile 10 %, basophile 1 %, lymphocytes 24 %, monocytes 4 %, plasma cells 0 %.

Sternal puncture (fig. 3): Culture abundant in cells. Myelopoiesis hyperplastic with most pronounced increase of small mature myelo-

cytes and metamyelocytes and relatively small excentrically located nuclei and acidophilous plasma. Nuclear construction coarse pitted, usually with a dark clump in the middle of the nucleus or at each end in lobed nucleus. Numerous rod nuclear leucocytes but extremely few segment nuclei. More than two segments not present. Increased number megakaryocytes and plasma cells. Erythropoiesis no remark.

Case 2. I. O. Journal No. 2317/45. *Diagnosis:* Polyneuritis post diphtheria. Pelger-Huët's anomaly of the nuclei of the leucocytes. Motorcar mechanic, 22 years old.

Heredity: Nothing known of interest.

Abusus and venerea denied.

Previously mainly healthy. 1944 producer-gas poisoning suspected. Treated at the producer gas policlinic, Sababtsberg Hospital, Stockholm. In good health after one month's rest.

Present illness: Diphtheria middle July 1945. At the beginning of August early symptoms of polyneuritis, on account of which patient was admitted to Södersjukhuset.

Status on 16/10—45: Polyneuritis symptoms; otherwise little of interest in status.

Blood: S. R. 4 mm/h. Hb 87 %. Red blood cells 4.54 mill. Index 0.96. White blood cells 5,000.

Diff. 16/10—45: Rod nuclear 24 %, segment nuclear 21 %, basophile 0 %, eosinophile 3 %, lymphocytes 42 %, monocytes 10 %.

Nuclei coarsely pillared, small. Two segments only.

Diagnosis: Pelger-Huët's anomaly of the nuclei of the leucocytes. Similar blood picture on re-examination 23/10 and 29/10 also on 12/11. Sternal puncture unfortunately not performed.

The patient was discharged on 16/11 with nerve symptoms in regression. On re-examination 15/1—46 subjective healthy. Nerve status no remark.

On investigation of family (fig. 4), the Pelger blood picture could not be found in parents or sisters and brothers. The patient has no children.

Case 3. A. J. Journal No. 3206/46. *Diagnosis:* Rheumatic fever? Pelger-Huët's anomaly of the nuclei of the leucocytes. 41 year old married woman, admitted during diagnosis: Hydrops gen. dx. et febris.

Heredity: Parents for the most part healthy. No rheumatic diseases in the family.

Soc. hyg. conditions: Nullipara. Menses no remark. Venerea denied.

Present illness: Middle April 1946 influenza with temp. 39° C. Subjectively healthy after 14 days. After having been up a couple of days, swelling in right knee and gradually also ache and pain on movement. Subfebrility. Admitted 9/5 1946.

Status: General condition good. Heart: systol. murmur on apex. Right knee: large exudate. Can bend knee only to 90°. Quadriceps-atrophy.

Blood: S. R. 32 mm/h. Hb 77 %. Red blood cells 4 mill. Index 0.96. White blood cells 4,400. Antistreptolysine titer normal.

Diff. 10/5—46: Metamyelocytes 2 %, rod nuclear 23 %, segment nuclear 25 %, basophile 1 %, eosinophile 5 %, lymphocytes 39 %, monocytes 5 %. Nucleus construction pachychromatic, mature, nuclei rather small and never more than two segments.

Diagnosis: Pelger-Huët's anomaly of the nuclei of the leucocytes.

Sternal puncture 17/5—46 (fig. 3). Culture moderately abundant in cells. In the myelopoiesis, increase of mature (acidophilous) relatively small myelocytes and metamyelocytes with a central dark clump in the nucleus, which is generally coarsely pillared (mature). Numerous peanut and dumb-bell shaped leucocyte nuclei. More than 2 segments do not occur. Megakaryocytes no remark. — Erythropoiesis no remark. Marrow picture typical for Pelger-Huët's anomaly of the nuclei of the leucocytes.

Patient discharged on 31/5—46, practically free of symptoms. *Diagnosis:* rheumatic fever rather uncertain, gonorrheal arthritis excluded.

On investigation into family (fig. 4) a sister displayed signs of Pelger leucocytes. Mother healthy. Father deceased.

Case 4. H. S. Journal No. S961/46. *Diagnosis:* Bronchial asthma. Pneumonia acuta. Pelger-Huët's anomaly of the nuclei of the leucocytes. Married woman, 54 years, admitted for pneumonia.

Heredity: Asthma 3—4 generations back on the mother's side.

Social and hygienic conditions: II-para. Menopause 1940. Has run the home since 1944, after having worked 19 years in a goldsmith's.

Previous illness: 1940 pneumonia treated at home, sulfa preparation and digitalis being administered. Since that time troubled by increasing asthma. 1941 increasing symptoms of heart failure, owing to which she gave up work in 1944. 1944 pneumonia again. Treated with sulfa. Course no remark. Same year again admitted to St. Erik's Hospital for hepatitis acuta. Differential count gave: rod nuclear 45 %, segment nuclear 21 %. No remarks in the journal respecting anomaly of nuclei of the leucocytes. 30/12—46 acutely ill with signs of pneumonia. Sulfa administered at home but patient admitted to Södersjukhuset 31/12 as she had grown worse.

Status: Pronounced dyspnoea, cyanosis of the face. Pulm: muffled and hard rattle in the right base and pleural rubbing sound. Fairly abundant ronchi and loose rattling. Heart: soft systolic murmur above the apex.

Lung X-ray 31/12—46: Parenchyma infiltration the size of a fist of pneumonia appearance in the upper dorsal part of the right lower lobe. Re-examination 16/1—47: The pneumonic congestion practically disappeared. No fresh changes. 1/2—47: No changes since 16/1—47.

Blood: 1/1—47: S. R. 109 mm/h. Hb. 77 %. Red blood cells 3.92 mill. Index 0.94. White blood cells 16,000.

Diff. (fig. 2): Myelocytes 29 %, metamyelocytes 47 %, rod nuclear 13 %, segment nuclear 3 %, eosinophile 0 %, basophile 0 %, lymphocytes 7 %, monocytes 1 %, plasma cells 0 %.

Strongly pronounced Pelger-Huët's anomaly of the nuclei. Nuclei small and coarsely pillared, structure mature. Toxic granulation.

Blood status: 10/1: Hb. 68 %. Red blood cells 3.6 mill. Index 0.94. White blood cells 25,000.

Diff: Myelocytes (immature) 2 %, metamyelocytes 18 %, rod nuclear 41 %, segment nuclear 24 %, eosinophile 2 %, basophile 0 %, lymphocytes 10 %, monocytes 3 %, plasma cells 0 %. (All eosinophile leucocytes round nuclear.)

Sternal puncture 8/1—47: Culture rich in cells. Myelopoiesis shifted to right with strongly increased number mature myelocytes with acidophilous plasma and small excentrically located coarse pitted nucleus. Few segmented leucocytes. More than 2 segments not present. — Megakaryocytes no remark. Erythropoiesis no remark. — Picture typical of Pelger-Huët's anomaly of the nuclei. — Megakaryocytes no remark. Reticule hyperplastic.

After the fever had subsided, blood status was taken on 20/1 that showed quite another picture: Hb 64 %, red blood cells 3.5 mill. Index 0.91 %. White blood cells 8,700.

Diff.: Metamyelocytes 2.5 %, rod nuclear 45.5 %, segment nuclear 18 %, eosinophile 2.5 %, basophile 0.5 %, lymphocytes 21.5 %, monocytes 8.5 %. The patient was afebrile on 16/1. S. R. had fallen to 64 mm/h. She was discharged on 3/2 in good state.

Re-examination of differential count on 20/5—47, or 4 months later, showed: myelocytes 4 %, metamyelocytes 19 %, rod nuclear 39 %, segment nuclear 5 %, eosinophile 3 %, basophile 0 %, lymphocytes 26 %, monocytes 4 %, plasma cells 0 %.

Investigation into family (fig. 4) showed existence of Pelger-Huët's anomaly of the nuclei of the leucocytes (see table) in two brothers, both childless, and a daughter and her 2-year old child. The patient's other daughter displayed normal leucocyte picture.

Discussion.

The most characteristic feature of Pelger-Huët's anomaly of the nuclei of the leucocytes is the pronounced discrepancy between the immature form and the over-mature structure of the nuclei. On differentiation solely according to nucleus form, the unsegmented leucocytes will be regarded as rod nuclear, metamyelocytes or myelocytes. The nuclei are noticeably short and broad (this, however, varies somewhat from case to case as may be seen from the micro-photographs) and they appear rather smaller than normal, so that the ratio between cytoplasm and nucleus is increased.

Leucocytes with more than two segments practically never occur. As a suitable name for the anomaly, we would suggest «false shift to the left».

The short broad nucleus with a waist-like indentation (peanut-shape) is extremely characteristic and never occurs except in false shift to the left. Specific to the Pelger picture also is the dumb-bell shaped nucleus, consisting of two globular segments with a short connecting thread.

The glass-eye shaped nucleus, also occurring in the anomaly, as is known, is to be seen also in normal blood — it is characteristic of the eosinophile leucocytes — and therefore is not pathognomonic of the anomaly. This shape of nucleus has occasionally been encountered in relatives (77 persons examined) of our patients and therefore has not been reckoned as sign of the person in question being »partial carrier» (bearer of both ordinary leucocytes and Pelger cells). Only leucocytes with dumb-bell or peanut-like nuclei have been taken as definite Pelger cells. As such leucocytes were not observed except in our full-carriers, it follows that we do not consider we can confirm statements in the literature that »partial carriers» might have been found in Pelger families.

As stated, the mature structure of the nucleus constitutes a strong contrast to the immature shape. The Pelger cell appears prematurely mature with its dark, coarse pitted nucleus and abundant acidophilous plasma. Nucleoli do not appear to exist in such cells. Nevertheless, according to the literature, they should frequently be present in Pelger cells. We have not been able to support this. We did, however, observe a dark chromatin clump in the centre part of the nucleus. This could not be nucleoli, as these of course are lighter than the surrounding nuclear substance.

The sternal puncture is characteristic. The more mature forms of myelopoiesis, myelocytes and metamyelocytes, are clearly increased. Here too the contrast between the immature form and the mature structure of the nucleus is striking. Whereas normally the myelocyte nucleus is appreciably lighter than the more mature cell nuclei, it is here dark and coarse pitted. Concentration of the chromatin to a dark clump in the centre of the nucleus is clearly apparent. The plasma also seems over-mature with increased tendency to acidophile. The nucleus is relatively small and often lies excentrically. Occasionally this feature is very pronounced.

Once one is acquainted with the false shift to the left, it is very easy to recognise in ordinary routine examination. In cases of high degree of shift to the left that is difficult to explain, a check examination is called for to exclude the false shift.

Table 1.

Table of Differential Blood Counts of 11 Cases of Pelger-Huët's Anomaly of the Nuclei of the Leucocytes.

Case	Remarks	Myelocytes	Neutrophile leucocytes			Eosinophile leuc.	Basophile leuc.	Lymphocytes	Monocytes	Plasmacells
			Metamyelocytes	Rod nuclear	Segment nuclear (2 segments)					
I T.L.	26/7-45	—	12	32	15	3	—	30	8	—
" "	6/8 "	—	11	35	11	1	—	33	9	—
" "	25/9 "	—	15	39	11	5	—	25	5	—
" "	17/6-46	1	3	46	11	10	1	24	4	—
E.L.	Brother to case I	—	1	30	33	2	2	28	3	1
A.L.	Grandfather to case I	—	2	32	18	1	1	38	8	—
II I.O.		—	—	24	21	3	—	42	10	—
III M.J.		—	2	23	25	5	1	39	5	—
A.E.	Sister to case III	—	—	26	35	3	1	26	9	—
IV H.S.	1/1-47	29	47	13	3	—	—	7	1	—
" "	8/1 "	2	18	41	24	2	—	10	3	—
" "	20/5 "	4	19	39	5	3	—	26	4	—
G.A.	Brother to case IV	18	28	25	2	1	—	18	8	—
A.A.	" " " "	2	12	25	21	2	1	32	5	—
M.L.	Daughter to case IV	2	5	27	2	3	1	50	8	2
Mona	Granddaughter to case IV, 2 years	—	2	32	18	—	—	38	10	—

Our case No. 4, strongly affected toxically when admitted, displayed during the acute phase a remarkable, almost grotesque blood picture. About one third of the leucocyte nuclei were round. At a later examination the changes due to the infection had disappeared, to be replaced by the customary Pelger picture.

In his case, Stahel was able with Pyriferr to develop a similar blood picture with »ultra-shift to the left».

Remarkable for our case No. 2 is that heredity investigation gave an entirely negative result. As has been stated, Stahel and Zündel have published cases where the usual course of heredity in Pelger-Huët's anomaly could not be demonstrated: neither of the parents was »full-carrier», though a number of the offspring were. Nachtsheim considered it probable that in these cases it was a question of a mutative occurrence of the Pelger gen.

Untersuchung der Kantharidenblase im Vorversuch den Kranken 3 Tage lang täglich 3×15 Tropfen Bellafolin (Sandoz) gegeben, nachdem sich uns dieses Präparat in früheren Ekg-Untersuchungen zur Dämpfung des Parasympathikus bewährt hat (Leitner und Steinlin). Da bei allergischen Reaktionen der Tonus des Parasympathikus erhöht ist, erschien uns diese Methode in Hinblick auf die Auffassung der KBR als allergische Reaktion (Kauffmann) aussichtsreich. Nach den 3 Bellafolintagen wurde erneut ein Pflaster aufgelegt und der Blaseninhalt nach 22 Stunden untersucht. Bei 7 derart untersuchten Kranken konnten aber keine verwertbaren Resultate erzielt werden, so dass eine Abhängigkeit der KBR vom veg. N. S. auf diesem Wege nicht nachgewiesen werden konnte.

Summary.

A series of 221 patients was investigated morphologically and biochemically by means of the cantharides blister. Eosinophilia in the blisters was found in 36 patients, most of whom also showed blood and marrow eosinophilia. Parallelism between the number of eosinophils in the blood and in the cantharides blisters could not be established. Eosinophilia existed in the blisters even when absent in the blood, and on the other hand in one case eosinophilia was absent in the blister though marked in the blood. It must therefore be assumed that tissue eosinotaxis plays an important part in the production of eosinophilia in cantharides blisters. Of the 36 patients 28 also showed an increase of lymphohistiocytes. 68 patients showed neutrophil polymorph leucocytosis, and in 78 the lymphohistiocytes were increased. 43 of the patients with disease processes belonging to Ranke's secondary stage showed lymphohistiocytic and 19 neutrophilic reactions. Among the patients with tuberculous cavities we found 38 with neutrophilic and 20 with lymphohistiocytic reaction. When the disease took a favourable course the reaction of the cantharides blister was often characterised by an increase of lymphohistiocytes, but these changes were not sufficiently frequent. We must therefore disagree with Kauffmann and conclude that the cantharides blister reaction fails to give guidance for diagnosis and prognosis in tuberculous patients, even when used in serial examinations.

The time-consuming technique of the morphological examinations precludes the popularization of the method of the

appear as if a high degree of shift to the left were present. It has not been possible for us to demonstrate any increase of nucleoli.

The sternal puncture displays a typical picture with increase of mature myelocytes and of metamyelocytes etc. The contrast between the immature form of the nucleus and its mature structure is also striking here. The plasma, too, appears over mature with a tendency towards the acidophilous.

Of the 4 cases described, case 1 was admitted for investigation owing to indefinite symptoms of the articulations and high sedimentation reaction, case 2 had post-diphtheric nerve symptoms, case 3 had a monoarthritis of indeterminate genesis and case 4 had pneumonia.

In the last-named case there was present during the acute phase an extreme shift to the left. About one third of the leucocyte nuclei were round. Later the customary Pelger picture appeared.

Pelger-Huët's anomaly of the nuclei of the leucocytes is inherited dominant. Among the 77 relatives of our cases that were examined we discovered 7 cases.

In the genuine Pelger cases (»full-carriers») all the leucocytes are of Pelger type. »Partial carriers» means cases having both normal leucocytes and Pelger leucocytes. Such cases might occur in Pelger families. Nevertheless, in the Pelger families investigated by us we found no »partial carriers».

It was possible in our cases 1, 3 and 4 to demonstrate the customary hereditary course, but not in case 2. This circumstance is discussed.

The practical importance of Pelger-Huët's anomaly of the nuclei of the leucocytes is pointed out. »False shift to the left» is proposed as a suitable designation.

References.

- Boström, L., Jonsson, E.: *Tidskrift för Sv. sjuksköt.* 7: 1947. — Dietzel, Karl: Inaugural-dissert. Erlangen 1935. — Huët, G. J.: *Klin. Wochenschr.* 30: 1264—66, 1932. — Jonsson, E., Boström, L., Bringel, B.: *Nord. Med.* 2051, 1947. — Kokubo: *Tokoku J. exper. Med.* 29: 519, 1936. — Leithner, St. J.: *Wien. Arch. inn. Med.* 37: 1943. — Leitner, St. J. and van Leeuwen: *Klin. Wochenschr.* 1: 1935. — Lorenz, Erich: Inaugural-dissert. Berlin 1937. — Nachtsheim, H.: *Der Erbarzt.* 11: 129, 1943. — Nachtsheim, H.: *Der Erbarzt* 10: 175, 1942. — Pelger, K.: *Ndld Tsch. Geneesk.* 72: 1178, 1928. — Schmidt, G.: *Zeitschr. Mikr.-Anat. Forsch.* 46: 459, 1939. — Stahel, L.: Dissertation,

From the Medical Department of Haugesund Hospital, Norway.
(Chief physician: Gunnar Benestad.)

Melorheostosis.

A Case with Biopsy.

By

SVERRE AARSETH.¹

(Submitted for publication November 17, 1947.)

Melorheostosis was described for the first time by the Frenchmen Léri and Joanny in 1922. The name is purely descriptive, and comes from the Greek words *melos*, limb + *rheos*, flow + *osteon*, bone. A roentgenogram of a long bone affected with this lesion, presents a picture resembling a dripping candle — «*en coulée de bougie*». It is a very rare disease. So far, to the author's knowledge, only a total of 35 cases have been published. Of these cases, one has been published by a Swede (Junghagen, 1930), two by Norwegians (Støren, 1936; Natvig, 1936), and one by a Dane (Bertelsen, 1940). In such a rare condition, every case published may yield further details to our knowledge of the disease.

At Haugesund Hospital we recently got the opportunity to observe an additional case of melorheostosis, the third one in Norway.

The patient is a woman, aged 40, the wife of a fisherman (Reg. no. 5742/46—47, B. G., b. May 5th, 1907). She was hospitalized in the Medical Department from May 19th till June 11th, and in the Surgical Department from June 11th till June 28th, 1947.

There is no history of malformations or deformities in her family. At the age of about 25, she noticed that there was an inspissation of the bone on the outer side of her right forearm. She had no subjective symptoms from her disease until 3½ years ago, when she got periodical, slight, but constant, irritating pains in the right forearm. She

¹ Ullevål Sykehus, Oslo.

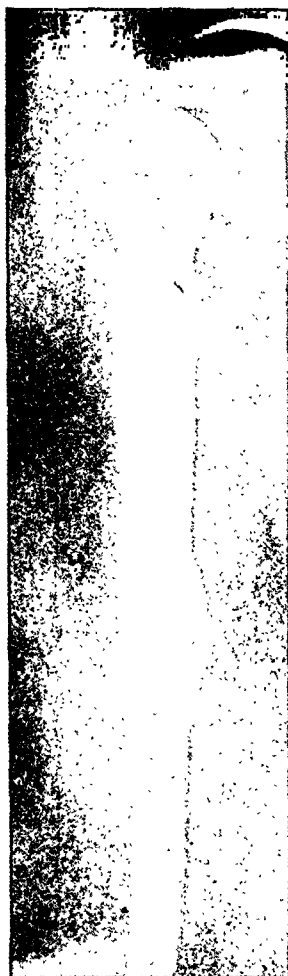


Fig. 1.

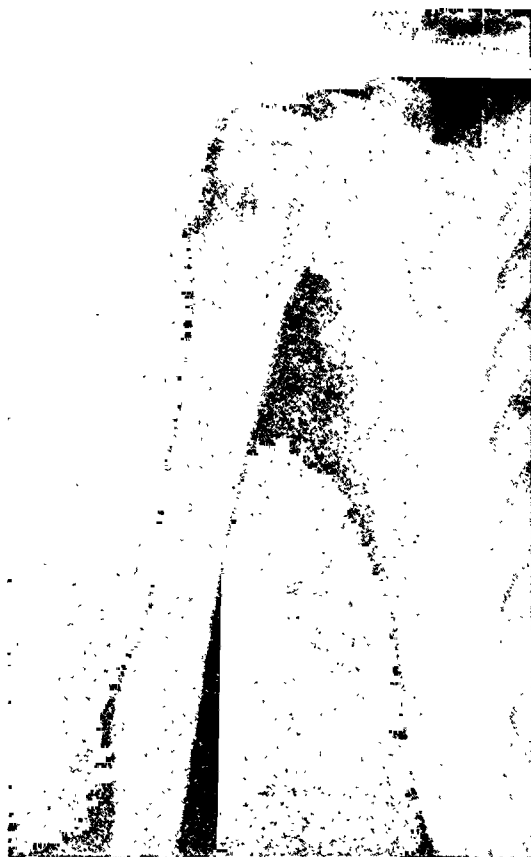


Fig. 2.

also noticed that it became difficult to rotate the lower arm and the hand outwards (supination). In October 1944, the arm was x-rayed by a private practitioner, but the nature of the disease was not recognized. Since then the symptoms have increased. The pains have never been intense, but strong enough to trouble her, especially on effort. She has also felt these pains on relaxing — »prickling and formication» — in the arm and in the right part of the chest. The limitation of supination has troubled her. She has a feeling of the right arm being colder than the left; the right hand easily becomes cold, and the arm becomes fatigued easily. In the beginning of May, 1947, she again consulted a physician, and she was referred to the Roentgenological Department (dr. K. Oppegaard) for examination. The roentgenological report runs as follows:

In the right humerus there is a streak-formed cortical thickening extending from the anatomical neck of the humerus downwards to the

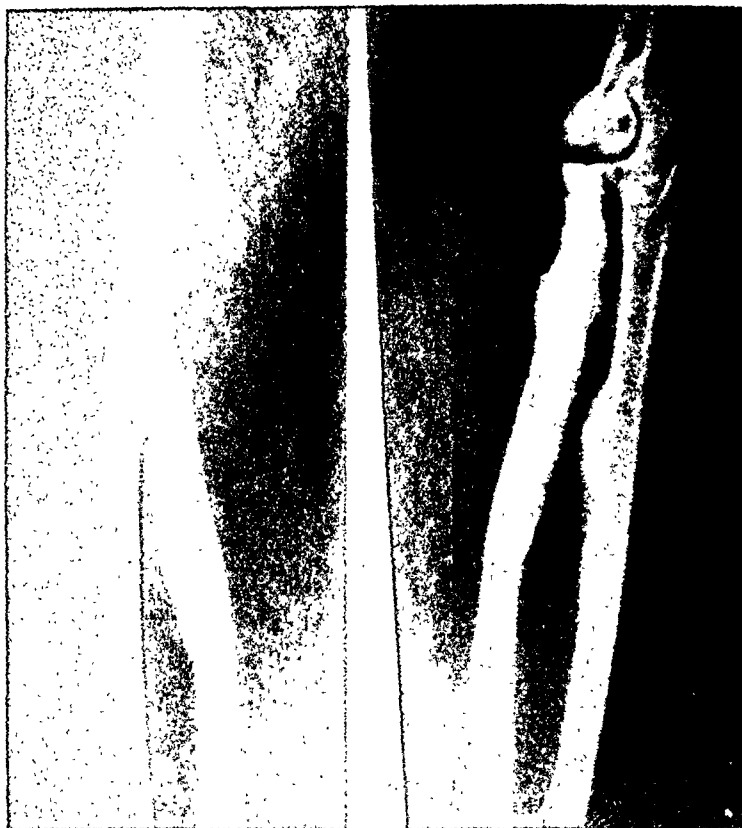


Fig. 3.

distal third of the diaphysis, the width varying a little, averaging about 1 cm. The zone is rather dense, and sharply circumscribed from the rest of the bone which has a normal consistency and shows regular bone structure. In the head of the humerus a couple of pea-sized, rather dense and homogenous, but irregular patches are seen.

In the proximal part of the radius (5—6 cm), several round and oval, pea-sized, or somewhat larger excrescences are seen, most of which are dense without evidence of bone structure. A more diffuse area is seen in the proximal part of the bone. The medullary cavity is somewhat narrowed. Furthermore, a streak-formed, about $\frac{3}{4}$ cm wide, cortical thickening extends distally to the middle of the diaphysis.

There is a cortical thickening of the middle part of the ulna.

The changes resemble those described in melorheostosis, but further roentgenological examinations of the skeleton ought to be carried out.

R: Probably melorheostosis. (K. O.).

The roentgenograms of the right upper extremity are presented in the figures 1—3.

Due to the roentgenological findings, indicating this rare disease, the patient was admitted to the Medical Department for further observation.

Physical examination, May 19th, 1947: The significant findings were located to the right upper extremity. The skin was normal, and no swelling or atrophy could be seen. There were free movements in the joints of the shoulder, hand, and fingers. Flexion, extension, and pronation took place normally in the elbow joint, but supination from the neutral midposition was impossible. Along the proximal third of the radius, a somewhat nodular, but smooth, bony hard, slightly tender thickening was felt. Similar findings in the proximal part of the humerus. There was no muscle weakness. Measurements: Acromion-olecranon, 31 cm (31 cm). Acromion to the tip of the styloid process of the radius, 57 cm (56 cm). The circumference of the wrist, 16 cm (16 cm). The circumference of the forearm 10 cm below the tip of the olecranon, 23 cm (22 cm). The circumference of the middle of the arm, 25.5 cm (24.5 cm).

Measurement of the skin temperature on the upper extremity with electrical thermometer revealed no difference in symmetrical areas.

Roentgenological examination of the skeleton did not reveal further abnormalities except for a spondylosis deformans incipiens. Roentgenological examination of the lungs, stomach, and duodenum was negative.

The roentgenograms of the right extremity were compared with those taken on October 4th, 1944 (dr. Nervik), 2½ years previously. The pathological changes were exactly the same, as well the appearance as the extent of the lesions.

Laboratory data: Pirquet 15–40 mm/48 hours. M. K. R. —. The urine did not contain albumin, pus, blood, or sugar. S. R. 3 mm/1 hour. Hb. 87 per cent. Otherwise normal findings in the blood, including a differential count. Icterus index (Meulengracht) 5. Serum albumin (Bing) 6.2 per cent. Takata-Ara —. Blood cholesterol 127 mg%. Calcium 9.7 mg%. Phosphorus 3.4 mg%. Phosphatase 3.4 units. Ewald's test meal: HCl/TA—0/5. The benzidine reaction in the feces was negative. The spinal fluid was normal. EKG: normal. B. M. R. 105 per cent. Glucose tolerance test: normal curve. Hormone titer (Nyegaard & Co. A/S Biologisk Laboratorium): Normal values for gonadotropic and estrogenic hormone, pregnandiol, and 17-keto-steroids.

The patient was considerably troubled by the restricted supination. The Surgical Department (K. Stray, M. D.) was consulted, therefore, and here an operation after the fashion suggested by Ingebrigtsen in the treatment of restriction of the rotatory motion of the hand due to badly healed fractures of the radius, was advised.

Hence she was transferred to the Surgical Department, and on June 16th, 1947, *resectio radii dext.* was performed (Stray). Corresponding to the outgrowth previously described, extraperiosteal resection of a 2.5 cm long piece of bone was made, this part with the outgrowth being considered as the direct cause of the limitation of the supination.

The part removed consisted partly of a bony hard, irregular, periosteal-coated tumor, the surface of which in some places had a glassy appear-

ance. A roentgenogram taken on a later occasion (fig. 4) showed that the proximal part of the bed, made by removal of the tumor, was 4 cm below the elbow joint.

The same day, after the operation, signs of lesion of the profund radial nerve, periferally to the branch to m. extensor carpi radialis,

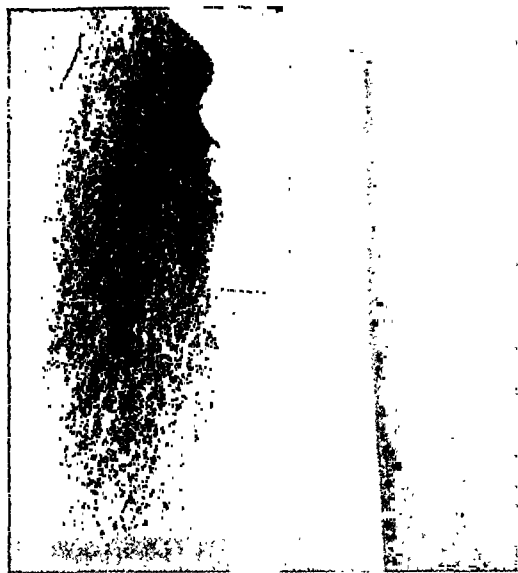


Fig. 4.

developed. An exploratory incision (Stray) was made the next day, therefore. A nerve was seen in the operational wound. The nerve was 2 mm thick, taut, swollen, and hyperemic, with a slight impression which probably had been caused by pressure during the operation. The continuity of the nerve was not broken.

The wound healed per primam. After the discharge from the hospital she has received physical treatment, exercise and massage. The neurologic symptoms have been retrograding.

On a control examination (Stray) on September 10th, 1947, there was no limitation of the movements of the wrist. From maximal pronation the hand could be supinated 135° (before the operation, 90°). Active movements of the four radial fingers were though still impaired.

The piece removed from the radius was sent to the Pathologico-anatomical Laboratory of Dr. F. G. Gade in Bergen for pathological examination, and the following report was received: The piece of bone has been decalcified. One portion of the specimen contains spongy bone with a rather fibrotic marrow. Otherwise the perparation consists of compact bone, the lamellae being arranged in concentric layers around a blood vessel (Haversian canal). Cartilage could not be found in the specimen. Diagnosis: Compact bone. (sign. Karl Bakken).

Comment.

The diagnosis of melorheostosis is purely roentgenological. The changes are so characteristic, that the condition cannot be mistaken for any other disease if one is only acquainted with it. In the vast majority of the cases described, the changes have been limited to one extremity only, or as in some instances, to the corresponding extremity arch. But this is no absolute rule. Bury (1939) found melorheostotic changes in the pelvis and both lower extremities in a 35 year old woman. Müller-Alberti (1941) found the process disseminated in all four extremities and in the pelvis in a 8 year old girl. Clément and Combes-Hamelle (1943) found the typical changes in the left arm, pelvis, and both legs in a 9 year old girl. Woytek (1933) diagnosed melorheostosis in a case where the changes were located to the lumbar vertebral column and to the first and second rib. Our case follows the general rule, the typical changes being located to one extremity only, the right, and here only in the long bones. In one case only (Bertelsen) the condition has been located to an individual bone, (right tibia).

The most important clinical *symptoms* in our patient are moderate pains in the extremity affected, paresthesias and fatigue in the limb, and limitation of movements. These are the usual symptoms. Limitation of movements in one or several joints is found in 50 per cent of the cases (Bertelsen). As a rule, the first symptoms appear before the growth is finished. Dillehunt and Chuinard, studying the literature, found that the symptoms set in after this period in 4 of 19 patients with melorheostosis. The clinical symptoms are commonly most pronounced in children, although the most extensive roentgenological changes are found in adults.

The *etiology* has been widely discussed. The incidence is equally divided between the two sexes. The disease is found in France, Italy, Germany, Poland, England, in the Scandinavian countries and in U. S. A. Kibby's patient was a Jap. Thus, there is probably no special race incidence.

Bertelsen is the first investigator who has demonstrated that heredity may play a part. In the mother of his patient he found changes in the left radius which might resemble melorheostosis. Furthermore, the right radius was abnormally curved. (This was

also found in one of Kraft's patients.) The brother of the patient had an abnormally curved right radius. As the disease is characterized by roentgenological changes only, and often do not give rise to clinical symptoms, it is evident that one will only occasionally get the opportunity to have the necessary examinations carried out in order to investigate the possibility of familiar occurrence. The information volunteered by the patient regarding heredity must be considered as worthless.

Goldschlag (1929) assumed that the lesion was due to endocrinous disturbances (thyroid? hypophysis?) but a definite hold for this does not exist. Our patient was especially examined taking hormonal disturbances into consideration, but the results were all negative.

Clément and Combes-Hamelle hold that melorheostosis and scleroderma are different manifestations of one and the same fundamental disease. The two conditions coincided in their case. This has also been observed by other investigators (Gillespie and Siegling, Dillehunt and Chuinard, Müller-Alberti. Meisel and Goldschlag found melorheostosis combined with Meige's disease). In scleroderma, hypercalcemia and tendency to calcareous deposits in the skin, subcutaneous tissue, and adjacent muscles (»hypercalcistie») is often found. In melorheostosis heterotopic formation of bone may occur (in 20 per cent of the cases — Bury). Disturbances in the calcium-phosphorus metabolism could not be demonstrated either in our patient or in previous cases where this has been especially investigated (Moore and de Lorimier, Gillespie and Siegling, Bertelsen).

Müller-Alberti, too, has drawn attention to changes in the skin and in the soft tissue (contracture and contraction of tendons) which might be associated with melorheostosis, and holds that all the changes may be easily explained as due to a common cause, such as an anomaly of the central nervous system. Putti suggests a combined neurovascular disease. A proof for these assumptions has not been established, nor has the theory of infection appeared to be of any real importance.

Most investigators now evidently incline to the views postulated by Zimmer, that melorheostosis is a congenital, developmental defect in a primitive segment. This theory easily explains all the changes which may appear, and also the fact that most of the cases may be traced back to the years of youth or childhood in spite of being stationary, or very slowly progressive.

Biopsy has previously been performed in 5 cases (Putti; Zimmer; Léri and Lièvre; Léri, Loiseleur and Lièvre; Junghagen). Common for all these examinations is the finding of compact bone with Haversian systems. In Kibby's patient, who had been operated twice, the operator found »very dense bone», but histological examination was not carried out. Some investigators have described a rarefying process besides that of the sclerotic one which characterizes the disease. Policard (in Putti's case) states that the sclerosis is secondary to the rarefaction.

The histological picture is in our case quite identical with that in previous cases: compact bone with Haversian systems, and fibrotic bone marrow. Evidence of infection or malignancy has never been demonstrated.

The treatment is purely symptomatic. Some of the patients will seek surgical assistance on account of deformities, contractures, or limitation of movements (Støren, Müller-Alberti, Kibby, the author's case). Radiation and diathermy have been employed to relieve the pains.

Summary.

The author describes a case of melorheostosis in a 40 year old woman who has been having subjective symptoms of her disease for 3½ years: pains, paresthesias, and limitation of movements. Roentgenologically, the disease has remained stationary for at least the last 2½ years. No evidence of endocrinous disturbances, changes in the calcium-phosphorus metabolism, or circulatory disturbances could be found. Histological examination revealed compact bone with Haversian systems, and a fibrotic bone marrow.

Literature.

Bertelsen, A.: Melorheostosis s. Osteosis eburnisans monomelica. Act. Chir. Scand. 83: 561, 1940. (With bibliography.) — Clément, R. and Combes-Hamelle, A.: Méléorhéostose et sclérodermie en bandes. La Presse Médicale 51: 311, 1943. — Franklin, E. L. and Matheson, I.: Melorheostosis; Report of Case with Review of Literature. Brit. J. Radiol. 15: 185, 1942. — Ingebrigtsen, R.: Behandling av rotasjonsinnskrenkning av hånden efter slett tilhelte radiusfrakturer. Norsk Magasin for Legevidenskapen 96: 963, 1935. — Kibby, S. V.: Melorheos-

tosis, with Report of a Case. Radiology 37: 62, 1941. — Müller-Alberti, W.: Ein Beitrag zum Krankheitsbilde der Melorheostose. Z.schr. f. Orthopäd. 72: 194, 1941. — Natvig, P.: A Case of Melorheostosis. Act. Radiol. 17: 498, 1936. — Nielsen, H.: Klinisk Endokrinologi. III. Kjøbenhavn 1942, pag. 278. — Støren, H.: Ein kasuistischer Beitrag zur Beleuchtung der Melorheostose. Act. Chir. Scand. 78: 94, 1946.

Centro de Investigaciones Fisiológicas, Buenos Aires.

Variability of the Lung Volume Measurements in Patients With Pulmonary Tuberculosis.

By

HUGO CHIODI.¹

(Submitted for publication October 23, 1947.)

Many papers have been written about the changes of the lung volume in normal and pathological conditions and also on those induced by the surgical treatment of some pulmonary illnesses. Notwithstanding and as far as we are aware, not much attention has been paid to the variability of the lung volume measurements on the same subject, when the tests are repeated in the same and different days, without any outwardly change in the thorax or lung conditions.

Bohr (3), Tobiesen (10), Christie (5), and others (1, 8, 9, 10) made several measurements of functional residual air or residual air in the same and different days, on a number of subjects. They averaged the results obtained in each individual and ascribed the mean variation found to errors of the method, without mentioning the possibility of physiological changes of the lung volume.

Cournand et al. (4) presented the results obtained in 134 subjects with the open circuit method; 158 duplicates of functional residual air determinations were made in the same morning, with a 15 to 30 minutes interval. The variability of their method was: in 74 duplicate tests or 46.8 per cent of the whole series, each determination deviated less than 2 % from the mean of the pair of values; in 109 duplicate tests this deviation was less than 3 %

¹ Now working at the Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires. Formerly Guggenheim Research Fellow in the Fatigue Laboratory at Harvard University.

from the mean; in 139 duplicates the deviation was less than 5 % and in 156 of the 158 cases the deviation was less than 7 % from the mean value of the pair.

G. Birath (2) made duplicate measurements of functional residual air on 14 normal and 8 pathological subjects using the hydrogen method. A calculation, according to Dahlberg, from the difference between the double determinations gave a S. D. for single determinations of ± 89 ml, S. E. ± 19 ml. The author did not observe any differences between normal and pathological cases.

According to Dahlberg «if a large number of determinations by a certain method are made on one individual, the values obtained are often distributed as a probability curve around a mean close to the mean of the individual. If only two determinations are made on this individual and the difference between those determinations is calculated, we have taken at random two values from such a probability curve and calculated the difference between these two random values. When another double determination is made on another individual by the same method, we choose in the same manner two random values from another probability curve of the same kind as the previous one. Accordingly, by continuing our determinations in the same manner we always choose two random values from a number of probability curves which are all alike, As a matter of fact, we obtain a series of differences conforming to the series of differences which would be obtained by choosing at random and repeatedly two values from one and the same curve.»

Therefore, the statistical treatment of a series of differences between duplicate measurements of functional residual air made on different subjects will permit us to calculate the variability, *i. e.* the error of the method used, since it does not seem reasonable to expect any real change in the lung volume of a resting subject in a 20 minutes interval.

On the other hand, if the differences from measurements repeated on the same subject within a few days period would be only due to errors of the method, such differences should not be greater than those found for measurements repeated in the same day. The existence of a greater variability from day to day than between the same day determinations would thus implicate the existence of changes due to other factors than the method's error, for instance variations of the reserve air, the residual air or both.

Subtracting from the variability of measurements made in different days the variability of the measurements made in the same day it would be possible to detect the existence of a real change in the residual air, that is, in the air remaining in the lungs after a forced expiration.

Experimental.

Functional residual air measurements were done with a modified Christie method already described in a previous paper by Izzo and Chiodi (7). Vital capacity, reserve air and complementary air were determined in a Benedict-Roth apparatus, the valves of which had been so adapted as to obtain the graphic tracing before CO_2 absorption. The procedure was as follows: with the spirometer filled with air and a small amount of oxygen the subject was connected with the closed circuit and a few normal breathings recorded. At the end of a normal expiration he was asked to continue the expiratory movement as much as possible. The measurements of the tracings from the end of a normal expiration to the maximal point of expiration gave the reserve air volume. Complementary air was measured from the end of a normal expiration to the point of maximal inspiration. For vital capacity determinations, the patient was directed to make at the end of a normal expiration a forced expiration followed by a maximal inspiration. The largest tracing of the three performed by the subject for each respiratory subdivision was considered as the real one.

Patients previously trained in the technique, came to the laboratory in the morning in fasting conditions and were allowed a half an hour rest before the experiment.

With the subject in recumbent position two functional residual air measurements were made in the morning with an interval of 20 minutes between each, to be sure, that all the oxygen had been flushed out from the lungs. At the end of the second measurement, reserve air, complementary air and vital capacity were measured as detailed above. The same procedure was repeated 1 to 3 days later.

For the sake of clearness we will call: A_1 , any of the two measurements of functional residual air made in the first day and A , the average of both; B_1 , any of the two second day measurements and B , their average; $(A-B)$, the differences between averages

of first and second day determinations; (A_1-A) and (B_1-B) the differences between any one of the two measurements of the same day and their correspondent average.

Results were calculated in ml, at 37 degrees C., saturated with water vapor and barometric pressure of the day.

On the whole, 297 duplicates of functional residual air measurements have been made on 117 lung tuberculous patients, 74 females and 43 males, without conspicuous emphysema and in a fair physical state. Some of them endured a thoracoplastie between two series of determinations.

The average body surface was 1.63 ± 0.17 sq. meters, the height 1.61 ± 0.03 meters and the mean age 23 years with a range from 16 to 61.

On 70 of the above mentioned patients, 53 females and 17 males, 96 double pairs of functional residual air measurements and the correspondent reserve air, complementary air and vital capacity determinations have been made.

The average values of the lung volume subdivisions were: functional residual air, 1,243 ml, S. D. ± 465 ; reserve air 308 ml, S. D. ± 183 ; complementary air, 1,555 ml, S. D. ± 552 ; vital capacity, 1,934 S. D. ± 596 .

Results.

Results of the 96 double pair tests of functional residual air and their corresponding reserve air and residual air volumes are grouped in table 1, although the ungrouped data were used for calculating our results. No sexual differences could be observed.

Differences between one of the two measurements of functional residual air or residual air made on the first day and the corresponding average (A_1-A), were calculated as percentages of such average. Similar figures for the second day differences, (B_1-B), were obtained,

The differences between averages of the first and second day measurements on the same subject ($A-B$), were calculated as percentages of the mean of the two averages.

Percentage differences grouped as detailed above are averaged in figure 1. It can be seen that the average of the differences between different days measurements ($A-B$) of functional residual air or residual air are greater than the averages of the differences between same day measurements (A_1-A) or (B_1-B). Statistical

Table 1.

Class limits of the differences	Class midpoint	Number of individuals				
		I	II	III	IV	V
30.1 -32	+ 31	—	—	—	—	1
28.1 -30	+ 29	—	—	—	—	—
26.1 -28	+ 27	—	—	1	—	—
24.1 -26	+ 25	—	—	—	—	—
22.1 -24	+ 23	—	—	1	—	2
20.1 -22	+ 21	—	—	1	—	—
18.1 -20	+ 19	—	—	—	—	1
16.1 -18	+ 17	—	—	1	—	4
14.1 -16	+ 15	—	—	1	—	—
12.1 -14	+ 13	1	1	3	—	—
10.1 -12	+ 11	1	—	1	1	5
8.1 -10	+ 9	2	—	8	2	7
6.1 - 8	+ 7	5	2	8	3	7
4.1 - 6	+ 5	8	12	13	6	12
2.1 - 4	+ 3	9	11	12	16	13
0.1 - 2	+ 1	22	23	13	8	10
0.09 - 0.09...	0	—	—	—	15	—
0.1 - 2	- 1	22	24	7	19	5
2.1 - 4	- 3	9	10	7	14	6
4.1 - 6	- 5	8	11	4	6	6
6.1 - 8	- 7	5	2	7	2	4
8.1 -10	- 9	1	—	2	1	4
10.1 -12	- 11	2	—	2	1	4
12.1 -14	- 13	1	—	1	—	2
14.1 -16	- 15	—	—	—	2	—
16.1 -18	- 17	—	—	2	—	1
18.1 -20	- 19	—	—	—	—	1
20.1 -22	- 21	—	—	—	—	—
22.1 -24	- 23	—	—	1	—	—
24.1 -26	- 25	—	—	—	—	—
26.1 -28	- 27	—	—	—	—	1
28.1 -30	- 29	—	—	—	—	—
30.1 -32	- 31	—	—	—	—	—

Column I — Differences between one of the two measurements of functional residual air made on the first day and the average of the pair, expressed as percentages of that average.

Column II — Same as above, for the second day measurements.

Column III — Differences between averages of the first and second day measurements of functional residual air, expressed as percentages of the mean of such averages.

Column IV — Differences between determinations of reserve air made on the first and second day, expressed as percentages of the mean value of the corresponding functional residual air.

Column V — Differences between residual air values of the first and second day tests expressed as percentages of the mean value of the corresponding functional residual air.

For statistical analysis, ungrouped data have been used; results given in the text being slightly higher than if grouped data would have been used.

treatment of the data as detailed in the following paragraph indicate that such differences are significant.

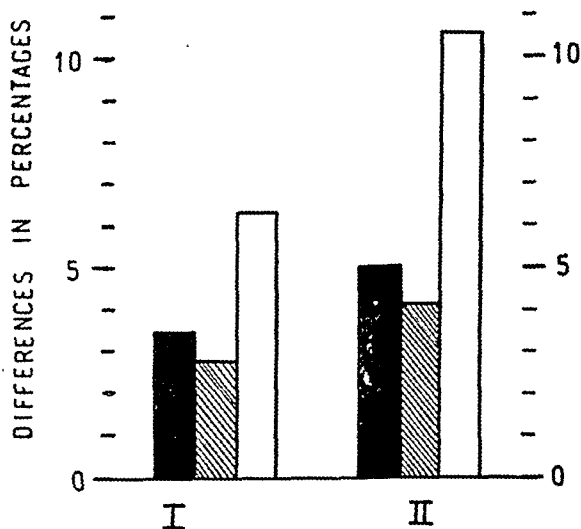


Figure 1.

Average of the differences between first day measurements (A_1-A).Average of the differences between second day measurements (B_1-B).Average of the differences between averages of first and second day measurement ($A-B$).

I Residual air.

II Functional residual air.

Statistical Treatment of the Data.

a) For the calculation of the standard deviation, σ , the following formula was employed:

$$\sigma = \pm \sqrt{\frac{\sum (d^2)}{N}}$$

where d = difference between averages of two double determinations or between the average of a double determination and any one of the pair.

When the real mean did not coincide with the 0 point around which the differences in percentages should be normally distributed, i. e. when there was an excess of positive or negative values, the following formula was used:

$$\sigma = \pm \sqrt{\frac{\sum (d^2)}{N} - b^2}$$

where b^2 represents the squared deviation of the mean from 0.

b) The standard deviation of a single experiment was calculated from pairs of determinations according to the following formula:

$$\sigma = \pm \sqrt{\frac{\sum (d^2)}{2N}}$$

where d = the difference between the two determinations of a pair.

c) The standard error, ε , of the standard deviation was calculated from the formula:

$$\varepsilon(\sigma) = \pm \frac{\sigma}{\sqrt{2N}}$$

d) The standard error of the mean was calculated according to the following formula:

$$\varepsilon(M) = \pm \frac{\sigma}{\sqrt{N}}$$

e) The standard error of the difference between standard deviations was calculated from the formula:

$$\varepsilon(\sigma_1 - \sigma_2) = \pm \sqrt{\varepsilon(\sigma_1)^2 + \varepsilon(\sigma_2)^2}$$

When differences between standard deviations exceed 3 times its standard error they are considered as statistically significant; differences exceeding 2.5 times their S. E. as statistically very probable.

f) According to Dahlberg if the total S. D. and the S. D. occasioned by one group of factors is known, the S. D. for the other group of factors can be found with the equation:

$$(\sigma_{1+2})^2 = (\sigma_1)^2 + (\sigma_2)^2$$

Functional residual air:

S. D., for $(A_1 - A)$ is ± 4.6 , S. E.¹ ± 0.33 ; for $(B_1 - B)$ is ± 3.6 , S. E. ± 0.26 ; for $(A - B)$ is ± 8.0 , S. E. ± 0.58 . Taking the first and second day values as a single series, $(A_1 - A) + (B_1 - B)$, S. D. is ± 4.0 , S. E. ± 0.21 .

The difference between the S. D. of A and S. D. of B does not exceed 2.1 times its S. E., therefore, it is not statistically significant.

The difference between the S. D. of $(A - B)$ and S. D. of $(A_1 - A) + (B_1 - B)$ exceeds 6.9 times its S. E., and is then statistically significant.

The differences between two measurements of reserve air made on the same subject with 1—3 days interval were calculated as percentages of the correspondent average values of the functional residual air volumes; the S. D. is ± 4.3 , S. E. ± 0.31 .

Subtracting, according to the formula indicated in f), from the S. D. of $(A - B)$ the variability due to errors of the method, *i. e.* S. D. of $(A_1 - A) + (B_1 - B)$, a value of $\pm 7.2 \pm 0.52$ is obtained, in which should be included any possible variation of the reserve air. The difference between the value ± 7.2 and the S. D. ± 4.3 of the reserve

¹ When not otherwise indicated it means the S. E. of the S. D.

air differences, exceeds 4.8 times its standard error being therefore statistically significant.

Subtracting from the value ± 7.2 the S. D. of the reserve air differences, a final value of ± 5.8 per cent of the volume of the functional residual air is obtained. This variability can only be explained on account of changes in the residual air volume.

The frequency curve drawn with the differences in percentages between functional residual air measurements made on the subject with 1—3 days interval, $(A - B)$, showed the values normally distributed around the mean ± 2.1 (S. E. of this mean ± 0.82) instead of around the 0 point, that is, there was an excess of positive values. The difference between the means being equal to 2.1, exceeds 2.6, its S. E., i. e. statistically very probable.

Differences were considered positive when the first day measurement was the higher one.

Considering the average value of a pair of measurements made in the same morning as a single determination the S. D. amounts to ± 5.4 , S. E. ± 0.4 per cent of the measured volume.

Calculating the S. D. of the 96 double pairs of functional residual air measurements in absolute figures, that is, directly in ml, the following results are obtained: for differences between determinations made in the same day, $(A_1 - A) + (B_1 - B)$, $\sigma = \pm 48$ ml, S. E. ± 2.4 ; for differences between measurements from different days, $(A - B)$, $\sigma = \pm 107$ ml, S. E. ± 7.4 . The S. D. of the averages of pairs of tests from the same morning considered as single determinations, amount to ± 76 ml, S. E. ± 5.2 .

The S. D. of the differences between the average and one of the two measurements of each of the 297 duplicates of functional residual air is ± 3.6 , S. E. ± 0.15 .

On the other hand, grouping that 297 duplicate tests as Cournand et. al. did, the following results are obtained: in 144 duplicates or 48.5 per cent of the total, each measurement deviated less than 2 per cent from the mean of the pair of values; 185 or 62.3 per cent deviated less than 3 per cent; 237 or 79.8 per cent deviated less than 5 per cent; 276 cases or 93.2 per cent deviated less than 7 per cent and in 287 cases or 96.7 per cent of the whole serie deviated less than 10 per cent from the average value of the pair.

Residual air. — The residual air volumes were obtained subtracting from each of the two functional residual air measurements obtained in the morning, the correspondent single determination of reserve air.

The statistical treatment of the differences between residual air measurements, calculated as percentages of the residual air volumes, gave the following results: the S. D. of the differences between residual air measurements made in the same day, $(A_1 - A) + (B_1 - B)$, was ± 6.1 , S. E. ± 0.31 ; the S. D. of the differences between measurements made in different days, $(A - B)$, was ± 13.4 , S. E. ± 0.97 . The difference between the two preceding S. D. exceeds 7.3 times its standard error, being therefore statistically significant.

Subtracting from the S. D. ± 13.4 , of the differences from different

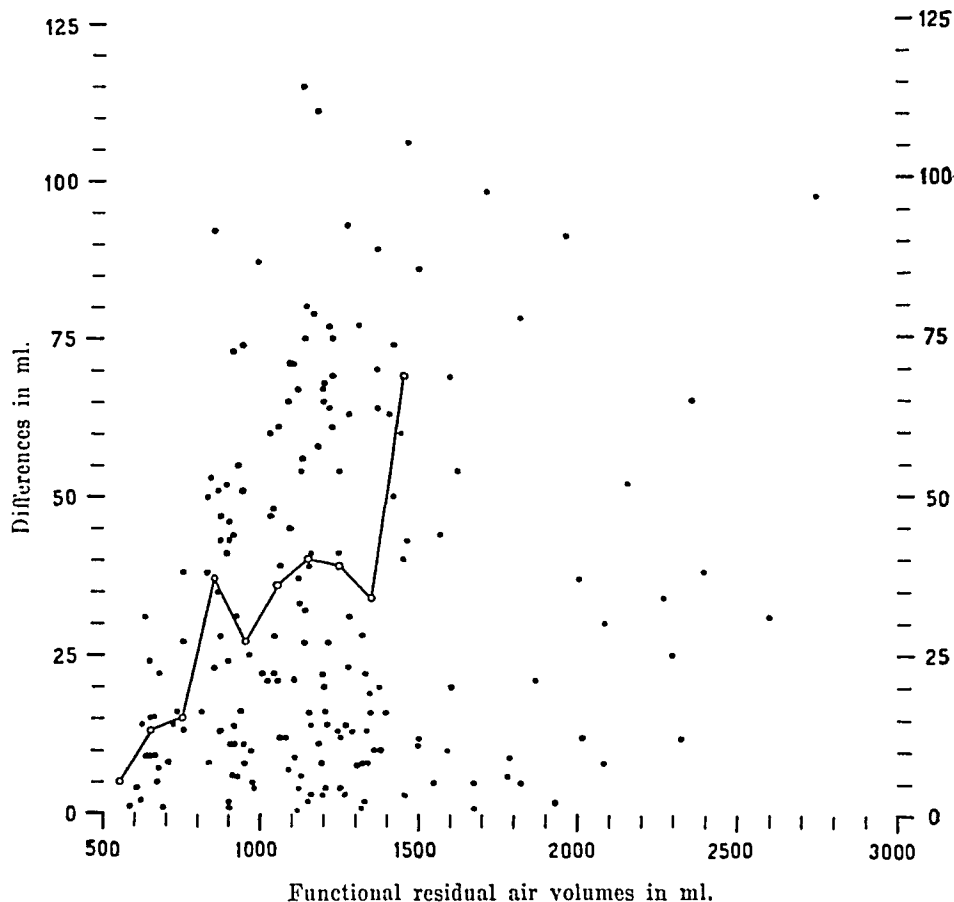


Figure 2.

On the abscissa: average volumes of the pairs of functional residual air measurements, in ml.

On the ordinate: differences, in ml, between one of the two measurements of functional residual air made in the same day and the average of the pair.

The circles connected by the full-drawn line represent the mean differences, calculated from the grouped data.

day determinations the S. D., ± 6.1 , of the differences from same day determinations, a value of ± 11.9 per cent of the residual air volume is obtained, which can be considered as the variability produced by changes in the residual air.

The S. D. of the averages of pairs of tests from the same morning, considered as single determinations, is ± 6.3 , S. E. ± 0.5 per cent of the functional residual air volume, or ± 9.1 , S. E. ± 0.7 , when calculated in per cent of the residual air alone.

Reserve air. — In the serie of 96 pairs of tests, the differences in ml between reserve air measurements made on the same subject with 1—3 days of interval gave a S. D. of ± 50 ml, S. E. ± 3.5 . Although there was an excess of values on the negative side of the distribution

curve, taken the 0 point as the mean, no statistical significance was found for that excess. The values were considered negative when the second day determinations were the higher ones.

The S. D. for single day determination amounted to ± 34.7 ml, S. E. ± 2.5 .

Vital capacity. — Measurements made under the same conditions as the reserve air gave a S. D. of ± 110 ml, S. E. ± 7.8 . For single day determinations the S. D. being ± 77.4 ml, S. E. ± 5.3 .

Although higher values of vital capacity were somewhat more frequent for the second day determinations than for the first ones, the differences were not statistically significant.

Complementary air. — S. D. of the differences in ml between measurements of complementary air made under conditions as mentioned above amounted to ± 116 ml, S. E. ± 11 . For single day determinations S. D. = ± 81 ml, S. E. ± 5.4 . No significant systematic differences between first and second day measurements were observed.

Discussion.

Studying the variability of the values of the lung volume in tuberculous patients, the absolute figures and their means have not been considered since the latter would not allow a true appraisal of the magnitude of measurements variations.

On the other hand, no statistical correlation has been observed between the functional residual air volumes of the subjects and the magnitude of the differences of measurements made either in the same or different days. On account of such lack of correlation it could seem unjustified to calculate the differences between pairs of determinations as percentages of their average volume. However, looking at figure 2 it may be allowed to say that there is a very rough trend of the means of differences to increase with the greater volumes. In fact, using the S. D. given in percentages for the calculation of the variability of the average of a pair of measurements of functional residual air, made in the same day, we found that for low or middle volumes the values obtained showed a better agreement with the range indicated by the graphic, than when the S. D. of the absolute difference in ml was used. For big volumes, the S. D. in percentages gives a too high variability.

Assuming for instance an average volume of functional residual air of 600 ml and another one of 1,400 ml, the S. D. for measurements in the same day being ± 4 per cent, the maximal variation would fall within 3 times the value of the S. D. or 12 per cent

of the respective volumes, *i. e.* ± 72 ml in the first case, ± 168 in the second one. If the correspondent S. D. of the absolute differences is used (± 48 ml) the maximal variation would be then ± 144 ml for both cases, therefore evidently too high for the lower volume, as can be seen in figure 2. For measurements made in different days somewhat similar results will be obtained.

As most of the volumes of the functional residual air of our tuberculous patients fall within the above mentioned values we thought justified the calculation of the S. D. in percentages.

For studying the changes of the residual air it may appear more logical to analyze directly their values instead of using those of the functional residual air, but as the residual air is calculated subtracting from the functional residual air the reserve air and the former shows an excess of higher values in the first day measurements and the latter in the second day, the differences from day to day would be in some cases artificially magnified. This is made evident by the higher results obtained, when in the calculations residual air instead of functional residual air differences were used, even if the former were calculated as percentages of the corresponding functional residual air volumes. Thus, to be in the safest side, functional residual air values have been preferably considered.

It was accepted that the variability found for the mean value of a pair of measurements of functional residual air, made on the same morning, was mainly due to errors of the method (poor mixture of the gases in the lung, uncertainty of the normal expiratory level at the start, miscalculation of the excreted nitrogen, etc.), since it is not likely to expect any real change in the lung volume of a resting subject, in a 20—30 minutes interval, except may be, in patients in whom the expelling of secretions could clear up a draining bronchiole of a pulmonary cavity or lobule.

Also, there is no reason to expect that on the same subject the variations of measurements due to the above mentioned errors of the method would be greater from day to day than on the same day. However, as the nitrogen percentage of the lungs of a subject can undergo changes, mainly conditioned by the depth and rate of breathing and in the calculations of the functional residual air a constant nitrogen percentage at the start of the measurements is assumed, some differences may result from day to day; yet, these differences must be quite small, since a variation of one per

cent in the lung nitrogen will only mean a change of equal magnitude in the measured volume. The thoracoplastie did not show any influence on the magnitude of the differences between different days tests.

On the other hand, differences between measurements made on the same subject in consecutive days would be the sum of the variability produced by the error of the method plus that induced by a real change in the lung volume.

The existence of such change is made evident when after subtracting from the total variability of the functional residual air volume that of the method, still remains a value, that in 2/3 of the cases does not exceed ± 7.2 per cent of the volume measured. It can be assumed now, that the variations found from day to day in the reserve air of a subject are real ones and not as Christie says, due to fortuitous changes in the expiratory muscular effort. In such case, the variations of the reserve air should be included in the S. D. or variability of ± 7.2 mentioned above. For that reason the S. D. of the differences between measurements of the reserve air made on consecutive days has been subtracted from the value ± 7.2 , resulting a S. D. of ± 5.4 per cent of the volume of the corresponding functional residual air. The variability of the reserve air has been calculated in percentages of the functional residual air, since any change of the former will appear as a modification of the volume of the latter.

On the other hand, if Christie's point of view is accepted, we must admit the constancy of the reserve air, which therefore would not induce any change in the volume of the functional residual air. The S. D. of ± 7.2 would represent thus the variability of the remaining component of the functional residual air, *i. e.* of the residual air alone.

In any case, it may be concluded from our results that at rest, the residual air, that is, the air that remains in the lungs after a forced expiration is not constant from day to day. The variations would not exceed ± 5.4 per cent of the corresponding volume of the functional residual air, in 2/3 of the cases.

An opposite point of view has been sustained by Christie, who thought it probable, that the inconstancy of the reserve air was mainly responsible for the variations found in the residual air of the normal individuals, as measured by his method. He disregarded the possible differences of the residual air of a subject from one day to another, given that he made an aver-

Undritz examined the blood from the parents in both these cases with the following results: In Stahel's case both the parents proved to be »partial carriers», the father of the usual type and the mother of the type described by Leitner where the leucocytes have coarse chromatin net and 3—5 round segments with thin linking threads. — In Zündel's case, the father at least was »partial carrier».

Nevertheless the hereditary conditions are not made clear by these finds, nor would Undritz suggest it. It should be strongly emphasised that »partial carriers» are not considered as bearers of the Pelger gen.

As stated, none of the relatives of our Pelger cases were »partial carriers», therefore including the parents of our case No. 2 — and this despite very careful examination of their blood. — The patient had no children.

Possibly in this case there may be question of a Pelger type that is new as regards occurrence. Illegitimate paternity might, however, provide the explanation. This possibility was considered to be excluded in Zündel's case.

Pelger-Huët's anomaly of the nuclei of the leucocytes, false shift to the left, has considerable theoretical interest, as has been seen. Undoubtedly, however, the practical importance is also great. If the anomaly is diagnosed, Pelger individuals would then of course be spared many complicated examinations and possibly also more or less risky or financially burdensome therapeutic interventions. — Consequently the false shift to the left requires to be known to the greatest extent possible.

At Södersjukhuset we have now had in the course of little more than a year 4 cases of false shift to the left. This indicates that this anomaly is not so uncommon. If a watch is kept out for the characteristic feature of the blood picture, these cases will probably be diagnosed to an ever greater extent.

Summary.

Pelger-Huët's anomaly of the nuclei of the leucocytes is characterised by a noticeable contrast between the immature form of the leucocyte nuclei and their mature structure. The nucleus seems small in relation to the cytoplasm. Dumb-bell or peanut shape in these is pathognomonic, but not the glass-eye form. It is exceptional for more than 2 segments to be formed. It would

lung subdivision, differences being in most cases almost the same either for small or big volumes.

The S. D. of the vital capacity is ± 77.4 ml; of the complementary air ± 81.4 ml and of the reserve air ± 34.7 ml.

Although a trend to show higher values on the second day measurements of vital capacity and reserve have been observed, air compared with those of the first day have been observed, the differences were not statistically significant. Therefore, no effect of training is evident when two determinations are made with 1—3 days interval.

Conclusions.

A statistical analysis of double pairs of functional residual air measurements and the corresponding single pairs of reserve and complementary air and vital capacity determinations made on lung tuberculous patients with 1—3 days interval, is presented.

1. — The residual air volume shows changes from one day to another within the range of ± 5.4 per cent of the corresponding functional residual air volume, in 2/3 of cases. They are not due to errors of the method or variations of the reserve air.
2. — The variabilities of the lung volume subdivisions, produced by errors of the methods and day to day changes, are given.

References.

1. Binger, C. A. L. and Brow G. R. J., *J. exp. Med.*, 39, 677 (1924).
- 2. Birath, G., Lung volume and ventilation efficiency. *Acta Med. Scand.*, Suppl. 154, 1944. — 3. Bohr, C., *Deutsches Arch. f. Klin. Med.*, 88, 385 (1907). — 4. Cournand, A., Baldwin, C. D. F., Darling R. and Richards D. W., Jr., *J. clin. Invest.* 20, 681 (1941). — 5. Christie, R. V., *J. clin. Invest.*, 11, 1099 (1932). — 6. Dahlberg, G., *Statistical methods for medical and biological students*. G. Allen and Unwin Ltd., London 1940. — 7. Izzo, R. A. and Chiodi, H., *Amer. J. Med. Sci.*, 206, 190 (1943). — 8. Mc Michael, J., *Clinical Science*, 4, 167 (1939). — 9. Peters, J. P., and Barr, D. P., *Amer. J. Physiol.* 54, 335 (1920). — 10. Tobiesen, F., *Skand. Arch. f. Physiol.*, 25, 209 (1911). — 11. Wolf, H. J., *Ztschr. f. d. ges. exp. Med.*, 62, 696 (1928).

From the IVth Medical Service of St. Erik's Hospital and the
State Bacteriologic Laboratory, Stockholm.

The Appearance of Acute Phase Protein After Induced Fever in Man.

By

PER HEDLUND, A. RUNE FRISK and HÄRJE BUCHT.

(Submitted for publication November 12, 1947.)

The diagnostic value of the presence of acute phase protein in various diseased conditions has recently been pointed out by Löfström (1) and Hedlund (2). They correlated the appearance of acute phase protein with temperature, sedimentation rate and white blood cell count, and found that rise in temperature and increase of sedimentation rate or of white blood cells do not run parallel with the formation of acute phase protein. Acute phase protein was present in the serum of all cases with a febrile temperature ($>38^{\circ}$ C.) while patients with slight elevation of temperature often show a negative reaction (2). It would be of interest, both from a theoretical and practical point of view, to know at what stage of the rising temperature the acute phase protein appears in the blood. We have therefore induced fever experimentally, and the appearance of acute phase protein has been correlated with the rise in temperature as well as with the white blood count and sedimentation rate, and with changes in the electrophoretic pattern.

Methods.

The capsular swelling reaction of Löfström (1) was used for the determination of acute phase protein in serum.

The experiments were carried out on seven afebrile convalescent patients with a negative capsular swelling reaction.

Fever was induced by a suspension of formalin-killed *Aerobacter aerogenes*, the stock solution containing 100 mill. bacteria

per ml. A diluted solution of this vaccine (2 ml per 1,000 ml of physiologic saline solution) was administered by continuous intravenous drip. The drip rate was regulated according to the rise in temperature, a constant temperature of about 39° C. being aimed at. Further details regarding this technique will be published (3). Blood samples for determination of acute phase protein, white blood cell count and sedimentation rate were taken immediately before the drip was instituted and then every third hour. The temperature was recorded every half or full hour. Usually when acute phase protein appeared in the blood in a titer of at least +2 the drip was removed and blood samples were taken less frequently, as a rule once every 24 hours until the temperature had returned to normal and the capsular swelling reaction was negative.

In two cases the changes in the electrophoretic pattern were followed.¹

Results.

Figures 1—3 are typical curves and show the behaviour of temperature, white blood cells, sedimentation rate and acute phase protein after continuous intravenous administration of

Case 2924/47 Diagnosis: duodenal ulcer

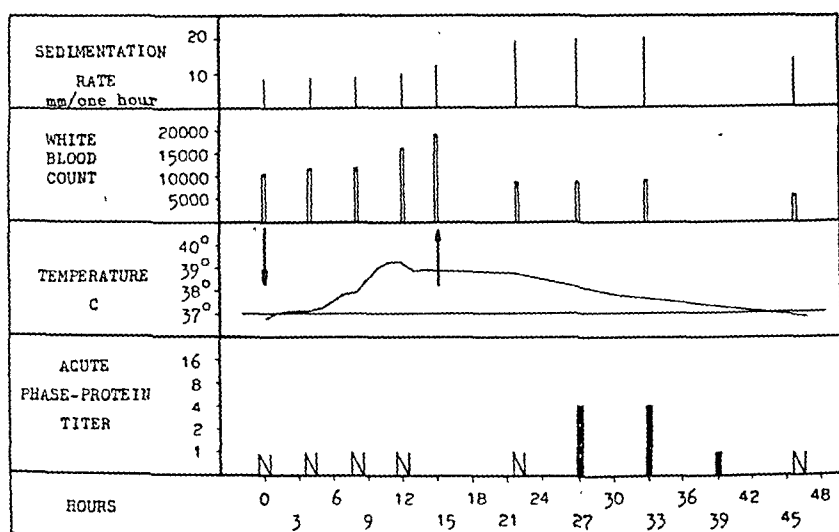


Fig. 1.

¹ The electrophoretic analyses were kindly performed by Dr. B. Olhagen. The buffer was composed of a phosphate buffer with an ion strength of 0.1, pH 7.6, plus 0.15 M NaCl, according to Svenson and Olhagen (4).

Case 3584/47 Diagnosis. duodenal ulcer

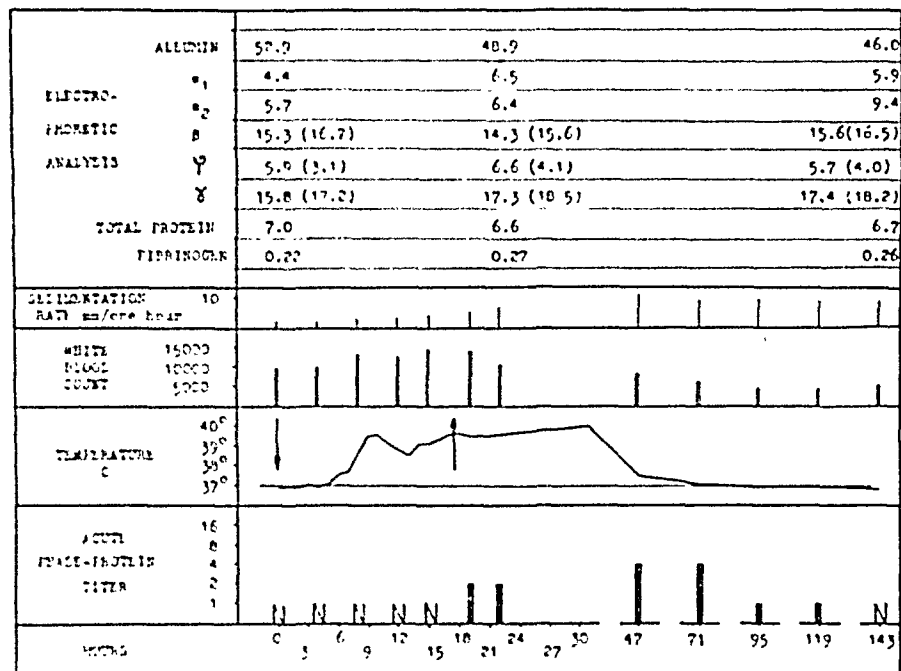


Fig. 2.

Case 3539/47 Diagnosis. duodenal ulcer

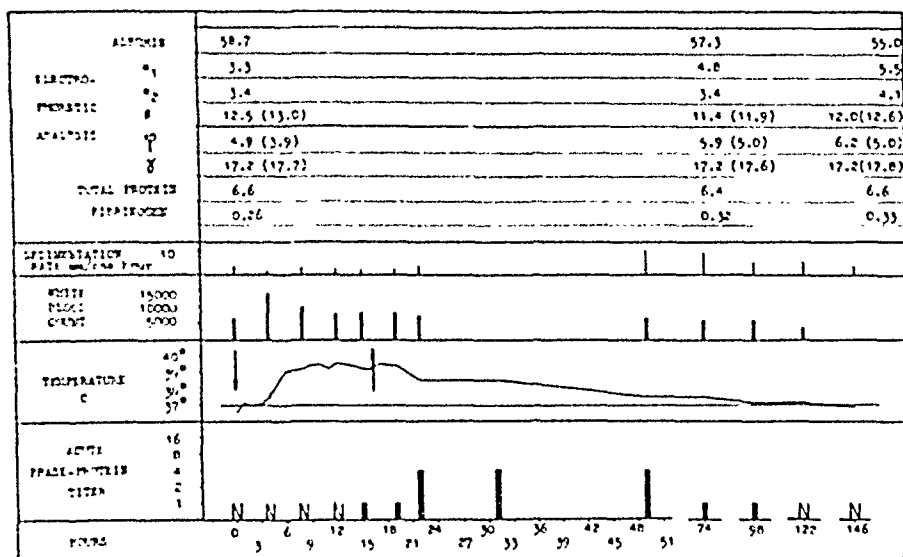


Fig. 3.

The figures of the electrophoretic analyses are expressed in percentage of the relative concentration. The figures within brackets have been corrected to agree with the *chemically* determined fibrinogen.

Aerobacter aerogenes vaccine. In all seven experiments the temperature began to rise from 5 to 7 hours after the start of the infusion. Simultaneously with the rise in temperature the number of white blood cells increased. As long as the *A. aerogenes* vaccine was being administered a leukocytosis was present in the peripheral blood. This subsided rapidly when the drip was suspended whereas the temperature took a longer time to return to normal.

Acute phase protein was demonstrated in the blood in six cases 15—21 hours, and in one case 27 hours, after the administration of *A. aerogenes* vaccine had been started. Thus, after the start of the temperature elevation, from 10 to 20 hours elapsed before acute phase protein appeared in the blood, the maximum increase being between 18 and 22 hours after. It disappeared from the blood at a slower rate, running parallel on the whole with the temperature drop; by the time the temperature had reached the normal value the capsular swelling reaction was also negative in most of the cases.

The sedimentation rate increased to a moderate degree in these experiments. The increase occurred late, and rise in the sedimentation rate was as a rule not observable until about 24 hours had elapsed after acute phase protein had appeared in the blood.

The electrophoretic analyses revealed a relative decrease in the albumin content and a relative increase in the globulins, the latter being almost entirely due to increased α -globulins.

Discussion.

With this experimental procedure it was possible regularly to induce fever after an interval of 5 to 7 hours. After a fever period of between 10 and 20 hours acute phase protein could be demonstrated in the blood. The point of time at which acute phase protein appears in the blood shows much greater variation in the individual cases than the time when the temperature elevation occurs. This may perhaps be explained by the fact that the acute phase protein was recorded less frequently than the temperature. The behaviour of temperature, white blood cells, acute phase protein and sedimentation rate after experimentally induced fever is similar to that occurring after myocardial infarction. In this condition, often with a sudden onset, there is a rise in temperature and white cells during the first day of illness. Acute

phase protein is always present in the blood and is demonstrable first on the second day after onset (2). The sedimentation rate increases slowly and a noticeable increase is not recordable until the third or fourth day of illness.

Electrophoretic fractionation has shown that acute phase protein in human serum belongs to the α -globulins (5). In our experiments, it was not possible to localize acute phase protein in the electrophoretic diagram. The increase in the concentration of α -globulins occurring when the titer of acute phase protein is at its peak (figs. 2 and 3) cannot be due to acute phase protein, since the increase was still observed, and was even more pronounced, in the samples taken after the acute phase protein had disappeared from the blood. In all probability, the increased globulin content may be interpreted as a non-specific reaction brought about by the bacterial protein. In favour of this view is the simultaneously increased fibrinogen, which in one case was significant (fig. 3). The reason why acute phase protein could not be located in the electrophoretic diagram is probably that it is present in the blood in such small quantities that it cannot be demonstrated by this method. A serologic method would therefore seem to be the best way of determining the acute phase protein.

Summary.

After experimentally induced fever of 10—20 hours' duration acute phase protein appears in the blood. The appearance of acute phase protein has been correlated with rise in temperature, white blood cells, and sedimentation rate and with changes in the electrophoretic pattern.

References.

- (1) Löfström, G.: Non-specific capsular swelling in *Pneumococci*. *Acta Med. Scand. Suppl.* 141, 1943. — (2) Hedlund, P.: The appearance of acute phase protein in various diseases. *Acta Med. Scand. Suppl.* 196, p. 579, 1947. — (3) Frisk, A. R. and Bucht, H.: *Nord. Med.* 37, 268, 1948. — (4) Olhagen, B.: *Acta Med. Scand. Suppl.* 162, 1945. — (5) Perlman, E., Bullowa, J. G. M. and Goodkind, R.: *J. Exp. Med.* 97, 77, 1943.
-

From the Medical Department of Maria Hospital, Helsingfors.
(Chief: Prof. Fredrik Saltzman.)

Alternating Nodal and Sinus Rhythm in a Case of Situs Viscerum Inversus.

By

RUBEN GORDIN.¹

(Submitted for publication November 17, 1947.)

Aschoff-Tawara's node receives, as is known, its impulses from the sinus node and sends them along the bundle of His to the bundle branch and thence to the ventricle walls. When there are disturbances in the higher placed centres or the paths of conduction, such as the sinus or sino-auricular block, or with physiological occurrences, sinus bradycardia, slow sinus arrhythmia, or during a pause after a premature systole, the atrio-ventricular node can take over the function of an impulse centre. It then sends out individual impulses so that the nodal escape or the nodal extrasystole arises, or also the node takes over the whole of the function, giving rise to a nodal rhythm. This is generally considered to be the sign of serious injury to the heart, but can also be the result of an intoxication due to different medicines. Magnusson quite recently reported in his publication, »Auricular standstill», two such cases after treatment with chinidin and digitalis. Such affections have been formerly demonstrated experimentally, in 1887 by Cushny in giving digitalis to dogs, and later by Lewis in using strophanthin, by Boden and Neukirch in administering chinidin etc.

In treating patients with these medicines, attention must be directed to the possibility of disturbances arising in the paths of conduction or in the centre, and in consequence of this, a nodal rhythm. Wolff and White assume, that when death occurs during a cure by chinidin, if it is not caused by emboli, it is due to the

¹ Minervagatan 1 C, Helsingfors.

standstill of the ventricle because the atrio-ventricular node is paralysed. This theory is supported by Korns' experiment on dogs, and calls for great care, especially when, in giving digitalis or chinidin medicine, an auricular fibrillation is transformed into a nodal rhythm.

The nodal rhythm arises generally as a passive function of the atrio-ventricular node when this, because the higher lying centres or leads do not work normally, must take over the function of an impulse centre. The following is a short description of a case in which the nodal rhythm seems to have arisen as an active function of the node of which the sensitiveness had increased.

The patient, an 83-year-old woman, formerly in the postal service, was in the Maria Hospital, Medical Department from 28. 9. 45 till 30. 1. 46. Diagnosis: Myodegeneratio cordis. Arteriosclerosis. Situs viscerum inversus.

The anamnesis showed nothing peculiar. She had been in good health generally, had pain in the legs for about a month and numbed feet so that it was difficult to walk.

Status: Unusually lively and alert for her age. Varices in the lower legs. Nothing noticeable about the nerve system and the lungs. Arteria radialis hard, uneven, twisting. No pulsation in arteria dorsalis pedis. Pulse even, regular except for occasional premature systoles. Pulse frequency reckoned at 40, 42, 44, 48 and 70/min. Heart on the right side, not enlarged. No murmur or accentuation. Blood pressure 180/100 mm Hg. Liver on the left, spleen on the right side. Urine: O. Hgl 82/95. WaR-X-ray: Situs viscerum inversus. Myodegeneratio cordis. Emphysema pulm. Ventricle lying to the right, colon ascendens to the left, colon descendens and sigmoideum to the right. Nothing else pathological in the intestinal canal.

Ekg 24. 3. 33 (Fig. 1.) In the first lead an ekg typical for situs inversus with a frequency of 75/min. Here the sinus rhythm suddenly changed into a nodal rhythm with a frequency of 40/min., later 50/min. Leads II and III show a sinus rhythm with a frequency of 75/min. We have thus in the first lead a sudden, temporary transition from a sinus to a nodal rhythm, which, however, quickly goes back to a sinus rhythm, which is visible in the later leads.

Ekg 1. 10. 45 (Fig. 2.) Lead I: The first four complexes are of nodal type, the rhythm nodal with a frequency of 42/min. (QRS = 1.4"). No 5 has an indication of a P-wave, the distance is shorter (1.2").

Lead II: The complexes Nos 1, 2, 4, 5 and 7 are of nodal origin, Nos

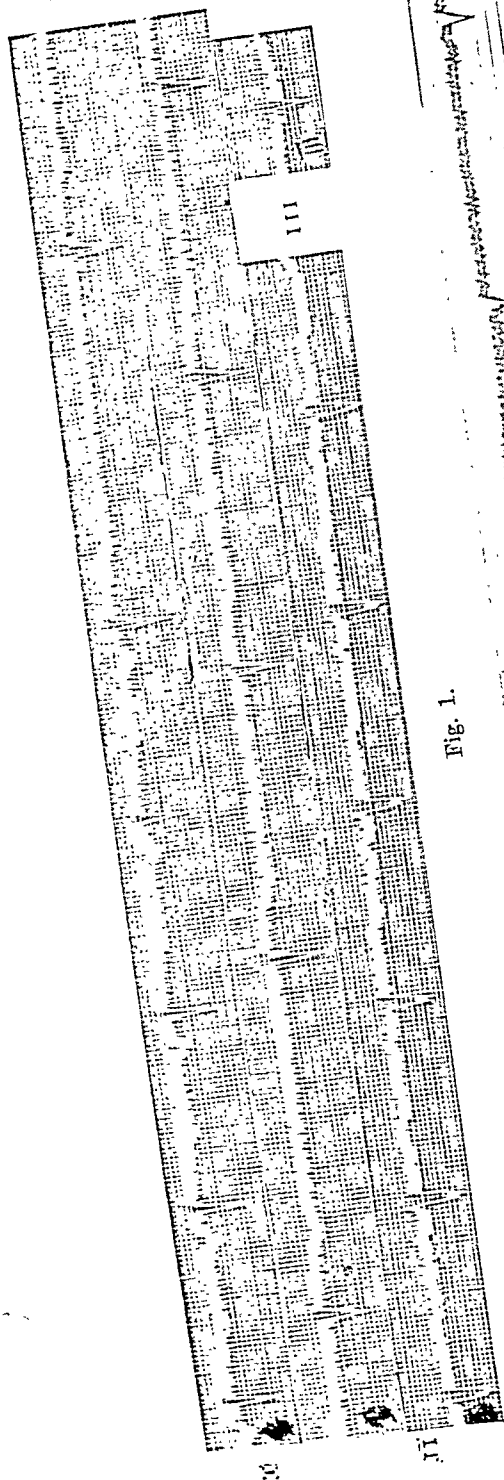


Fig. 1.

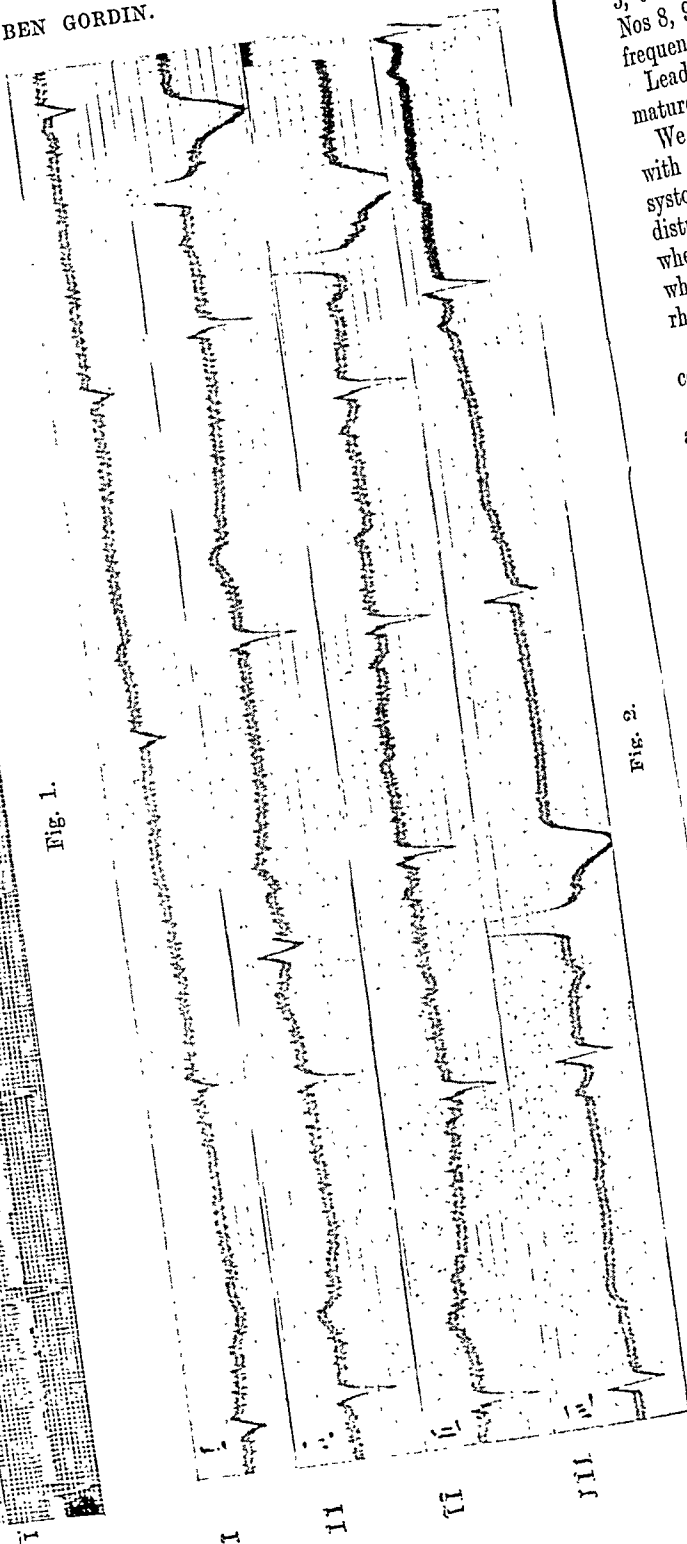


Fig. 2.

3, 6 and 12
Nos 8, 9 and
frequency of
Lead III
mature sys-
We have
with one,
systoles of
disturbance
where the
while a
rhythm.
Ekg
contin-
Ekg
a fre-
press-
this
the
can
from
L
ti

3, 6 and 12 are ventricular premature systoles of different origins. Nos 8, 9 and 10 proceed from the sinus node, a sinus rhythm with a frequency of 60/min. PQ 0.15".

Lead III: Nos 1 and 4 are of nodal origin, No 3 is a ventricular premature systole, Nos 2, 5 and 6 are sinus complexes.

We have here an *ekg* in which the middle nodal rhythm alternates with one, led from the sinus node; besides these ventricular premature systoles of different origin. The question is therefore one of severe disturbances in the leads, we have clearly here a sino-auricular block, where the sinus impulse is at times led to the atrio-ventricular node, while again there is at times a blocking, causing the rise of the nodal rhythm.

Ekg 8. 10. 45 shows a nodal rhythm in leads I and II which at first continues in lead III and then transforms into a sinus rhythm.

Ekg. 26. 8. 45 (Fig. 3): Here we have a regular nodal rhythm with a frequency of 37/min. Instead of a slow rhythm, the sinus carotid pressure gives a more rapid rhythm with a frequency of 63/min., and this rhythm originates in the sinus node. After three sinus complexes the rhythm again becomes nodal, with the same frequency as before the carotid pressure. Thus instead of a vagus effect we get one, proceeding from the sympathetic, because the patient is irritated by the pressure. Later tests with carotid and bulbar pressure have, with one exception, given the same result.

After exertion (Fig. 4), a sinus rhythm appears in leads I and II, in lead III a nodal rhythm with a frequency of 46/min. The carotid pressure brings back the sinus rhythm which is transformed into a nodal one with a frequency of 37/min.

Ekg 13. 11. 45 shows a nodal rhythm with a frequency of 46/min.

Ekg 21. 1. 46 shows a regular sinus rhythm with a frequency of 55/min., which by means of the carotid pressure is transformed into a regular nodal rhythm with a frequency of 41/min. When the pressure ceases, the nodal rhythm continues.

Summary of the Electrocardiographic Changes.

These show a nodal rhythm alternating with a sinus rhythm, the latter usually arising with exertion, or when the patient has been exposed to some kind of interference which irritates her. Thus repeated sinus carotid pressure, except for one occasion when the sinus rhythm becomes a nodal rhythm which continues after the pressure has ceased, does not cause any diminishing of the heart's frequency but, on the contrary, a quicker heart action, that is, a sympathetic effect. Here there is clearly a sinus node with a lowered capacity for sending impulses (the patient is 83 years old with definite sclerotic changes), and it appears most often to function when the heart is subject to greater strain.

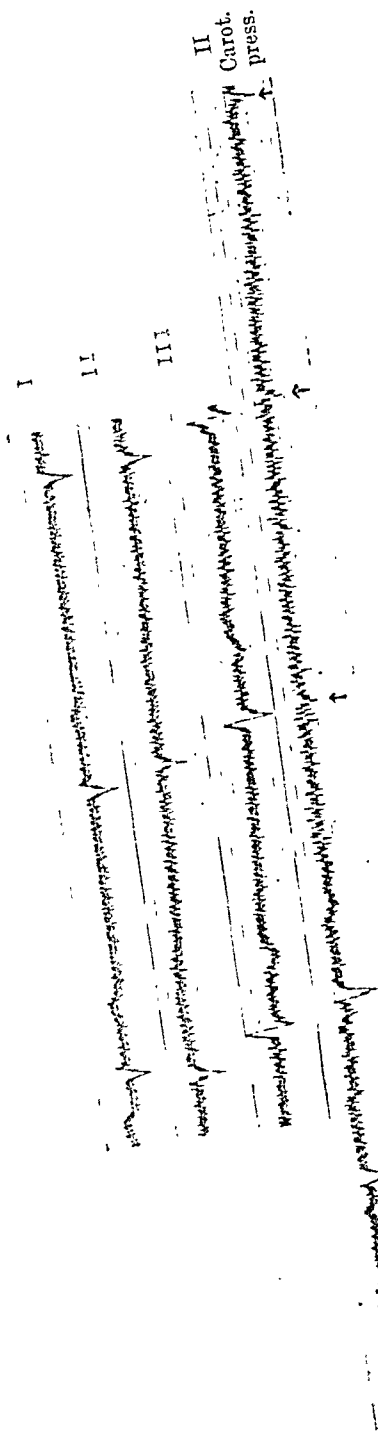


Fig. 3.

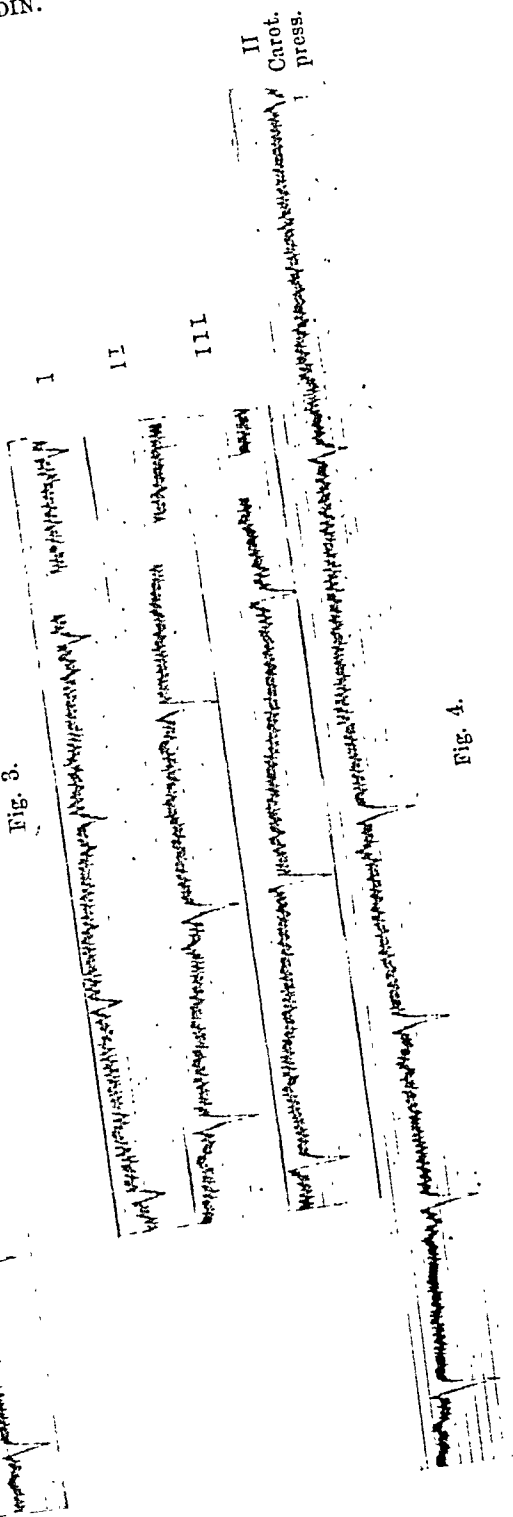


Fig. 4.

The nodal rhythm which so often shows itself, a function of the atrio-ventricular node of which the rhythm alternates with one proceeding from the sinus node, points to a certain disposition of the Aschoff-Tawara's node to take over the function of an impulse centre actively, and it must be assumed, that this nodal rhythm is, at least partly, the result of an increased irritability in the node, as an active rhythm, and not, as in generally, as a passive one due to the fact that the impulses of the sinus node have disappeared.

A special phenomenon, which to a certain extent indicates grave disturbances in the heart action, deserves mention. In ekg from 1933 T I was negative as it usually is in a case of situs viscerum inversus. In the later ekg:s the T I was positive. We may probably take this positive T I as an inverted T, and as of the same importance as a negative T I in ordinary heart conditions, and as a sign, that in process of time, sclerotic changes will occur in the heart's musculature. I have not seen this phenomenon mentioned in the literature, still less commented upon.

Theoretically atropine should speed up the heart action, but according to Scherf and Boyd an opposite effect is found in 30 % of normal people, although of temporary nature. »Atropine produces an 'inverse' excitation of the vagus which is evident for a short time before the paralytic effect becomes apparent.» This has also been pointed out by Eekl, Wilson and others, and is explained by the fact that atropine does not paralyse all vagal endings simultaneously, but that for a short while the atrio-ventricular node takes over the radiating of impulses. A similar test with our patient (Fig. 5) shows this effect, though of temporary nature, in that the nodal rhythm only occurs in lead I, to give place in the others to the original sinus rhythm. This test too may possibly support the conception of the atrio-ventricular node's tendency to radiate impulses.

The patient returned to hospital at the beginning of September, having had a sudden attack of apoplexy with right-sided paralysis. A few weeks later exitus letalis. At the post-mortem a total situs viscerum inversus was confirmed. In the left corpus striatum a yellowish-brown softening focus was found the size of a little finger tip. The heart weighed 400 g and looked quite normal except that the position of the chambers was exactly the reverse of what is usual.

The heart muscle uniformly greyish-white without foci, the

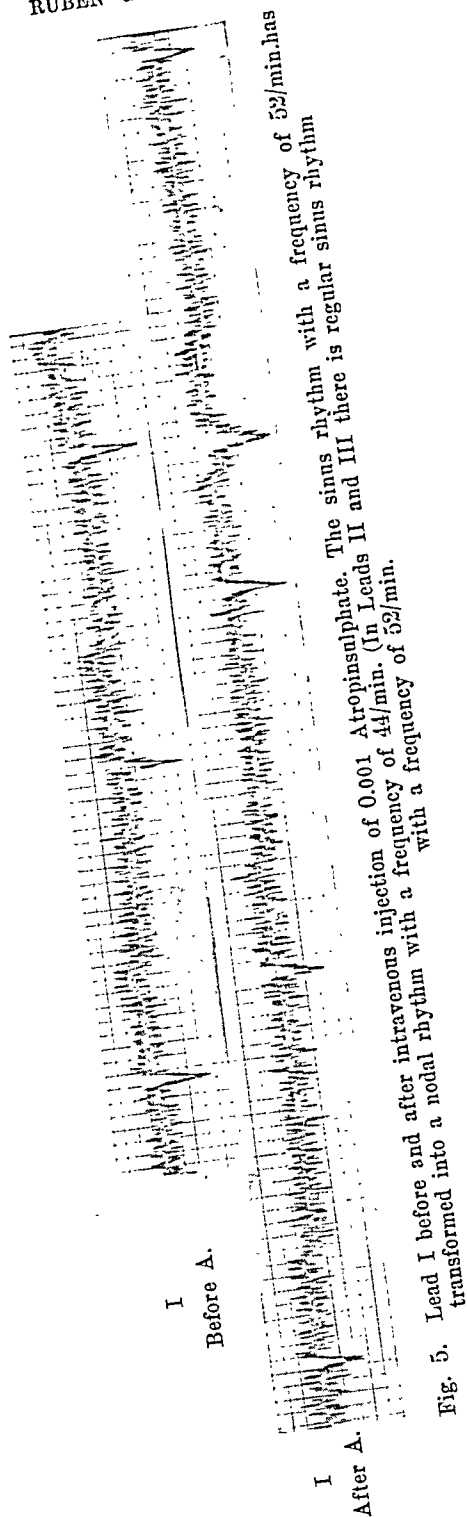


Fig. 5.

valves showed nothing remarkable. The right auricle much dilated. The caecum long and loose and on the left side, colon ascendens and colon descendens both on the right side. On the right side were 4 rather large and some small spleens, all close together but clearly separated. Arteriosclerosis changes in the aorta. In the main branch of the right arteria pulmonalis was a greyish-red, easily loosened embolism as thick as a finger. Kidneys finely granulated with a weakened cortex. Post-mortem diagnosis: *Situs viscerum inversus. Embolia rami dx. art. pulm. Nephrosclerosis. Hypertrophia cordis. Arteriosclerosis.* Histological examination of the heart and blood vessels showed extensive sclerosis, not least in the interventricular septum. By an oversight the histological preparation was not safeguarded *lege artis*, and therefore a more detailed examination of the heart's specific conduction system could not be made.

Summary.

First a short description of Aschoff-Tawara's node and its function as the impulse centre at different junctures.

The case reported shows electro-cardiographic changes in the form of a rhythm alternately nodal and sinus, the latter usually when the patient had been subjected to some overexertion. Thus, repeated sinus-carotid pressure did not result in a bradycardia but, on the contrary, in a more rapid heart action due to an irritation of the patient. It seems as if the ability of the sinus node to send out impulses was lowered. The nodal rhythm which so often appears — a function of the atrio-ventricular node — which rhythm alternates with the one from the sinus node, indicates a certain disposition in Aschoff-Tawara's node to actively take over the function of an impulse centre, and it must be assumed, that this nodal rhythm is, at least to some extent, a consequence of an increased sensitiveness in the node — that is, an active rhythm — and not, as in general, a passive one due to the impulse of the sinus node having disappeared.

Literature.

Boden, E. & Neukirch, H.: Dtsch. Arch. klin. Med. 1921: 136: 181. — Cushny, A. R.: J. Exp. Med. 1887: 2: 233. (Ref. Magnusson). — Eckl, K.: Wien. med. Wschr. 1919: 69: 440. — Korns, H. M.: Arch. Int. Med.

1923: 31: 15. — Lewis, T.: Quart. J. Med. 1913: 6: 221. — Magnusson, P.: Acta Med. Scand. 1946: 123: 519. — Scherf, D. & Boyd, L. J.: Clinical Pathology. London. 1945. — Wilson, F. N.: Arch. Int. Med. 1929: 43: 653. — Wolff, L. & White, P. D.: Arch. Int. Med. 1929: 43: 653.

From the Internal Department of Leyden University Hospital,
The Netherlands.

Simplified Micro-Hirst Technique.

By

Professor Dr. J. MULDER and Dr. W. R. O. GOSLINGS,
assisted by Miss S. W. ENSERINK.

(Submitted for publication December 1, 1947.)

In 1941 Hirst (1) found that red cells of the hen are agglutinated by the influenza virus. In consequence of this discovery the technique of the virological and immunological investigation of influenza has become simplified to such a degree that it has become available for every clinical laboratory, provided the influenza virus strains are placed at its disposal.

The principle of the Hirst technique, as modified by Salk (2), is as follows. For the titration of a certain quantity of influenza virus a series of twofold virus dilutions in saline is mixed with a 0.5 per cent (Hirst) or a 0.125 per cent (Salk) final dilution of red cells. The highest dilution that still shows a four plus agglutination is a standard for the titer of the virus (not for the virulence, as killed virus also shows this phenomenon). For the titration of antibodies in blood serum a series of twofold serum dilutions is mixed with a constant quantity of influenza virus and red cells. Where the virus is neutralized by the serum, no agglutination is observed.

This technique requires a very large number of tubes and pipettes and for quick working also automatic pipettes. The reading is only reliable after one hour and a half, when the agglutination-pattern has subsided at the bottom of the tubes. As in the case of human sera one must always work with two virus strains (A and B), 50 % of the tests yield negative results.

The following modification gave us much satisfaction as it saves time, glass ware and biological material. It was developed

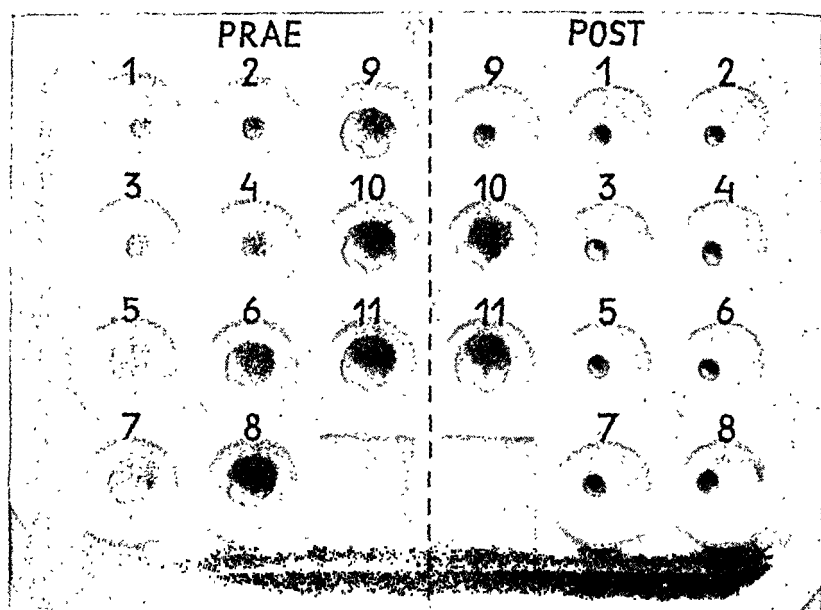


Fig. 1. Immune-body ferretserumtitration before and after infection. Influenza-hemagglutination test in a tile. The first serum-dilution is 12.

in consequence of a communication from Goslings, who published the same technique for a rapid micro penicillin-titration for clinical use (3).

Instead of tubes, small dye-trays (tiles) are used, the concavities of which are examined beforehand to see if they will yield good sedimentation-patterns of the red cells. The diameter of the holes is 11 mm and the greatest depth 9 mm. If the holes are too steep the agglutination-pattern often shows a collapse which sometimes interferes with an exact reading. The same phenomenon is often seen when using the macro-method according to Hirst.

Dilutions of virus and serum are prepared with a Pasteur-pipette. The final dilution of the red cells must be 0.5 per cent, else the pattern of agglutination and sedimentation becomes indistinct. The agglutination-pattern is very beautiful and its formation can be easily followed. Owing to the slight quantity of fluid in the concavities the settling process is complete within half an hour. After a quarter of an hour one is able to read it. The tests can be made with one drop of virus solution, for instance amniotic or allantoic fluid of inoculated eggs and one drop of serum. In the case of the agglutination inhibition test with immune sera or patients' sera mixtures of serum dilutions and virus

are kept one hour at $+4^{\circ}\text{C}$ before adding the red cells, as we found that the first agglutination-patterns were less clear when the red cells were added at an early stage (Fig. 1). All tests are done in the ice-box at a temperature of $+4^{\circ}\text{C}$.

Criticism of the Method.

The only objection to this method is the dropping, which is not quite equal, according as saline, serum or red cells are used. The preparation of the dilutions with the Pasteur-pipette is also liable to a certain error. These errors are slight, however, and in a comparative examination of the sera of patients they are not of importance. Generally speaking we use the method for the preliminary examination of a large number of patients' or ferret sera. We always use the method for a rapid determination of the titre of amniotic and allantoic fluids from inoculated chick embryos.

Summary.

When applying the Hirst technique for the titration of influenza virus and serum antibodies one can save time, glass-ware and materials by using small dye trays (tiles) with concavities, instead of glass tubes. Both the agglutination and the sedimentation pattern may be seen quite clearly and beautifully after half an hour.

References.

1. G. K. Hirst, *Science*, 94, 22, 1941. — 2. J. E. Salk, *Journal Immunol.*, 49, 87, 1944. — 3. W. R. O. Goslings, *Nederlandsch Tijdschrift voor Geneeskunde*, II, 1542, 1947.
-

From the Clinic of Internal Diseases, University of Cracow (Poland).
(Director: Prof. Dr. Tadeusz Tempka.)

Normal and Pathological Lymphadenogram in the Light of Own Research.¹

By

Prof. Dr. TADEUSZ TEMPKA and Doc. Dr. MIECZYSLAW KUBICZEK.

(Submitted for publication November 3, 1947.)

Micromorphological examination of the smears of material obtained by the puncture of pathological lymphatic glands, *i. e.* the preparation of lymphadenograms, is a great advance in modern clinical methods of investigation. This method of biopsy, in spite of its undisputed value, has not been up to now universally applied. The most important cause for the lack of the general application of lymphadenograms is the difficulty of correctly evaluating the picture.

In evaluating the morphological picture of lymphadenogram one must take into consideration two points: first the estimation of the general character of the morphological elements, second the evaluation of their relative proportion. One must stress that sometimes even this method of investigation is not sufficient for diagnosis and one must base it on the other investigations and the whole clinical picture.

At the beginning it is necessary to mention the advantages and disadvantages of the examination of smears from aspirated glands. First material for examination gained in this way is, to a certain extent, obtained blindly, as in every other similar method, and in small amounts. Second, through aspiration the specimen is separated from its physiological surroundings and smeared on a slide, it is not surprising therefore that the picture appears to be quite

¹ The work was finished just before the war and could not appear. As there was no possibility to continue our research work under the German occupation we publish this paper only now, with a few new cases.

gram, namely the red corpuscles which through retrogressive changes are losing gradually the property of oxyphilic staining for basophilic one. There was a complete lack of erythroblasts.

4) The Thrombocytes.

No typical thrombocytes were observed either single or in groups. They were looked for especially in the fields of vision with fewer cells and free from detritus, as otherwise it would be difficult to distinguish them in the background filled with concretions, even if they were present. No megakaryocytes or megakaryoblasts were seen.

A small number, about 0.5 per cent of the cells or nuclei observed cannot be recognized and placed in any of the above described groups. Occasionally cells in stage of mitosis were seen that also could not be definitely classified.

On the basis of the above bio-morphological data of a normal lymphadenogram the following conclusions may be drawn concerning the productive and destructive activity of the lymphatic gland with reference to the morphological elements of the blood. *The productive activity* has two aspects: first the production of lymphocytes, which is proved by the presence of their young forms *i. e.* lymphoblasts, second — the production of reticular cells, which appear singly or more often in groups in every normal lymphadenogram and show a tendency to phagocytosis. On the other hand there is no evidence of erythro-, granulo- and thrombo-cyto-blastic function in the normal lymphadenogram. *The destructive activity* includes first of all lymphocytes which split and disintegrate usually extracellularly, but it must be emphasized that lymphocytolysis occurs partially also through phagocytosis by the reticular cells. We also confirmed the occurrence of scanty erythrocytolysis. That the red corpuscles are destroyed in the lymphatic glands is supported by the presence of the degenerated basophilic erythrocytes showing the remnants of oxyphilia as described above. The presence of the «naked nuclei» of the reticular cells and the splitting off of the cytoplasm of these cells is evidence of their destruction. That we have to do with the pictures of actual destructive activity of the gland and not with the artifacts is supported by that in the same fields of vision, and therefore subject to the same external conditions, we find different corresponding cells that are very well preserved.

Comparing our findings with those of other authors (E. Weil, I. Wall and S. Perlès; Fleischhacker and Klima; Stahel) we are in complete agreement with their description of a normal lymphadenogram. The French investigators define normal lymphadenogram as »banal et uniforme constitué en majeure partie par des lymphocytes adultes». The above mentioned Austrian and Swiss authors give the following composition of a normal lymphadenogram: the majority of cells are adult lymphocytes, single lymphoblasts and reticular cells; in the reticular cells the remnants of phagocytosed cells are found; of other forms one finds red corpuscles, plasma cells and neutrophil polymorphonuclears. The composition as given by these authors differs from ours only in that we did not corroborate the presence of plasma cells in the normal lymphadenogram. No data concerning the production or destruction of cells in the normal lymphatic gland were given by the above mentioned authors.

Lymphadenogram in Pathological Conditions.

In research on lymphadenogram of glands that have been changed by disease, our problem was to determine whether, and to what extent, this method can be used for diagnosis, analogically to the use of the myelogram and splenogram. For our research we took smears of enlarged lymphatic glands from cases in which the diagnose was certain and as a control we examined the glands histologically in sections. Our material comprised 70 cases grouped as follows: lymphadenoma (Hodgkin's disease) — 32 cases, tuberculous glands — 18 cases, chronic aleuemic lymphadenosis — 3 cases, chr. leuemic lymphadenosis — 5 cases, acute myeloblastic leucemia — 2 cases, chr. myeloid leucemia — 3 cases, neoplastic glands — 7 cases.

Lymphadenoma (Hodgkin's disease) gives a series of different appearing lymphadenograms, which however have one common characteristic namely the polymorphism of the cell elements. This polymorphism is created by the following cells:

A) Cells not appearing in a normal lymphadenogram: 1) Sternberg's cells, 2) monocytes and monocytoïd cells, 3) plasma cells, 4) eosinophils. We must accentuate once more that according to our findings the cells named under 2), 3), and 4) never appear in a normal lymphadenogram.

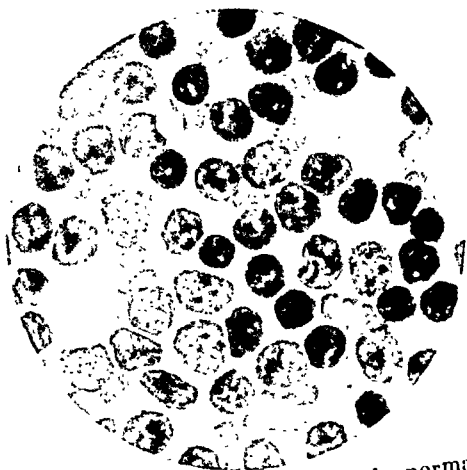


Fig. 1. Lymphadenogram of the normal lymphatic gland.



Fig. 2. Lymphocytes in the initial stage of disintegration.

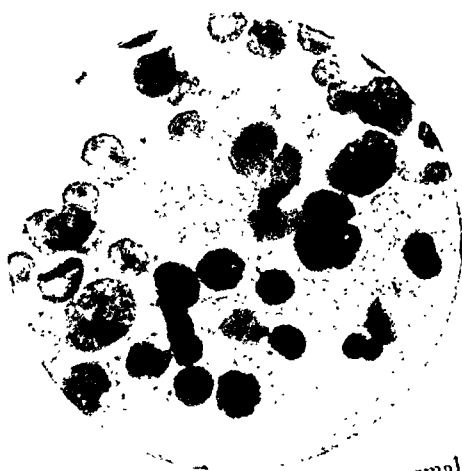


Fig. 3. Reticular cells in the normal lymphadenogram.

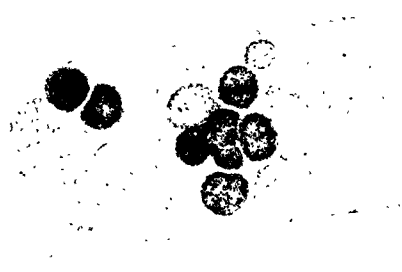


Fig. 4. Erythrocytolysis.

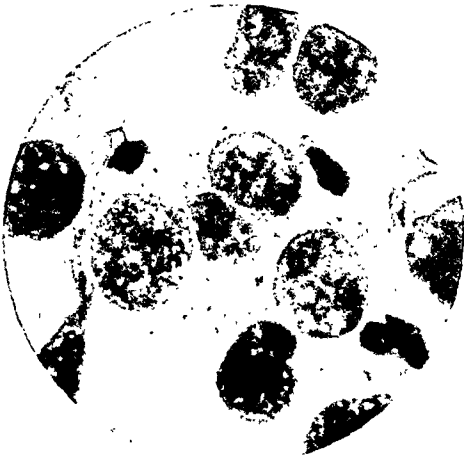


Fig. 9. Transitional forms from reticular to Sternberg's cells.

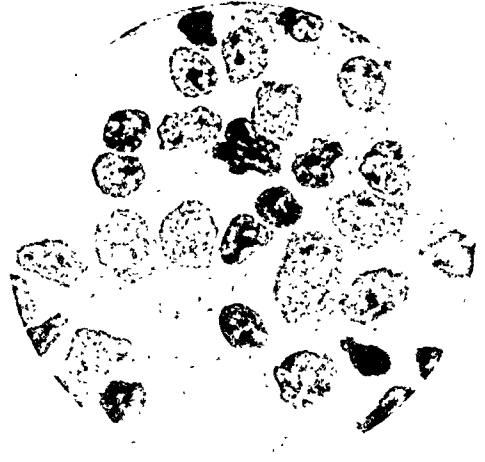


Fig. 10. Increased proportion of lymphoblasts in the case of chronic lymphatic leucemia.

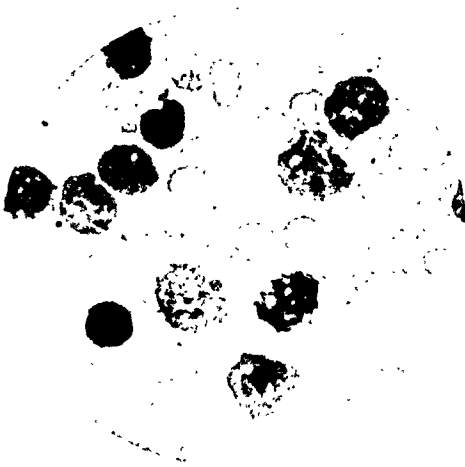


Fig. 11.

Fig. 11. Gumprecht's shadows.

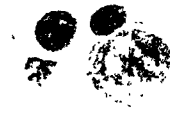


Fig. 12

Fig. 12. Myeloblast in the lymphadenogram of a chronic myeloid leucemia.



Fig. 13.

Fig. 13. Myeloblasts in the lymphadenogram of an acute myeloid leucemia.

Tuberculous Glands.

The lymphadenograms obtained in 18 cases of tuberculous cervical lymphatic glands gave the following results: In 8 cases the picture was completely normal, apart from increased number of lymphoblasts and reticular cells in some fields of vision. In the remaining 10 cases enlarged, hard, non-fluctuating glands were punctured and the smears showed a completely formless detritus, in which more or less numerous neutrophil granulocytes and lymphocytes could be seen, some of them with well preserved structure while others were in a state of disintegration. We did not observe in any case either giant cells or Koch's bacilli. Because of scanty material gained by the puncture we did not inoculate guinea pigs. With regard to the diagnostic value of lymphadenogram in tuberculous glands it is concluded that there are no special morphological characteristics on which to base the diagnosis, unless of course it is possible to show the presence of Koch's bacilli or at least giant cells, neither of which were seen in our preparations.

Leucemia.

Our material comprises 5 cases of chronic leucemic lymphadenosis, 3 cases of chronic aleucemic lymphadenosis, 3 cases of chr. myeloid leucemia, and 2 cases of acute myeloblastic leucemia.

Chronic leucemic lymphadenosis: The erythrocytes are very much the same in number and appearance as in the normal lymphadenogram. There are no granulocytes. The lymphocytes fill every field of vision and the proportion of lymphoblasts in comparison to the normal lymphadenogram is greatly increased (Fig. 10), sometimes to such an extent that they outnumber the adult forms. Here and there cells in stage of mitosis were observed. »Gumprecht's shadows» are more numerous than in normal gland. The lymphoblasts have on the whole a typical appearance; single cells however are a little larger, and show a similarity to reticular cells in their structure. They seem to be transitional forms between lymphoblasts and reticular cells. The number of reticular cells, together with Türk's cells, are greatly increased; endothelial cells were not observed.

Chronic aleucemic lymphadenosis (Fig. 11). The most characteristic is the great number of Gumprecht's shadows, outnumbering

age of several measurements repeated on the same subjects in different days.

The residual air changes may be considered as due, in part at least, to variations in the extension of the functioning alveolar fields and in the blood filling of the lungs. Those changes of residual air would induce a corresponding variation on the resting respiratory level, if a concomitant modification of the reserve air does not take place. Obviously we are dealing here with the constancy or inconstancy of the resting expiratory level of the thorax, which according to Wolff (11) shows a tendency to become fixer in the pulmonary tuberculosis. The point is not well established yet, and it will require further studies. It must not be forgotten, however, that in tuberculous patients an occlusion of a bronchiole by secretions could temporarily exclude from the active gas circulation a cavity or a number of alveoli with the consequent modification of the residual air volume.

Before going further in the discussion it would be interesting to have similar data from normal subjects.

Such variability of the residual air volumes must not be disregarded in studying the changes produced on the lung volume by the surgical treatment of cancer or tuberculosis of the lungs.

For judging the significance of a change of the mean value of the functional residual air, in pulmonary tuberculous patients, measured by duplicate and with the method employed by us, the S. D. of ± 5.4 per cent of the volume measured should be used.

For residual air the S. D. of ± 9.1 per cent of its volume or ± 6.3 per cent of the volume of the functional residual air should be used.

The analysis of the 296 duplicate measurements of functional residual air made on pulmonary tuberculosis shows, that the results secured with our technic agree fairly well with those obtained by Cournand et al. with their open circuit method.

The variability of the average of our duplicate measurements of functional residual air as single determinations are much lower than that obtained by Birath, S. D. ± 48 against ± 89 ml. The greater volumes of his series, which determinations were made in sitting position, could perhaps account for the difference.

S. D. of single determinations of vital capacity and complementary and reserve air have been calculated in ml instead of percentages of their volumes, on account that no correlation has been observed between variability and volume magnitude of the

form, cannot in itself ascertain the diagnosis, as qualitatively it does not differ from that of the normal gland, and a definite increase in the number of lymphoblasts and reticular cells is not yet a sufficient proof. As regards myeloid leucemia, both acute and chronic form, a definite myeloid metaplasia can be easily seen in the lymphadenogram. The acute form can be differentiated from the chronic one by fewer adult granulocytes in the former. However, in estimating the value of lymphadenogram for diagnosis of myeloid leucemia, one must remember that myeloid metaplasia in the lymphatic glands may appear apart from leucemia also in other diseases as was confirmed by different authors. Thus Herzenberg found local myeloid metaplasia of the cervical glands in diphtheria, scarlet fever, and measles. In cases where there were slight changes in peripheral blood, characterized by an increased number of leucocytes and the presence of single myelocytes, Hirschfeld confirmed myeloid metaplasia of glands and spleen in the course of such infections as diphtheria, scarlet fever, erysipelas, lobar pneumonia, meningitis, tuberculosis of the lungs, and other general infections. Concluding, lymphadenogram showing myeloid metaplasia has diagnostic value only when taken into consideration together with the whole clinical picture.

It should be emphasized that our research seem to show the existence of transitional forms between reticular cells on the one hand and the lymphoblasts and myeloblasts on the other. This fact suggests that reticular cells may develop, at least in pathological conditions, into lymphoblasts or give the origin to myeloid metaplasia of the gland. The findings of Fleischhacker and Klima and of Stahel are in complete agreement with ours: in cases of lymphatic leucemia they corroborated the increase of the cell elements of the normal lymphatic gland, and in myeloid leucemia they found myeloid metaplasia of the lymphatic glands.

Malignant Neoplasms.

Seven cases of carcinomatous glands were examined: 4 cases of carcinoma of the lung with metastases in the supraclavicular and cervical glands, 1 case of cancer of the hepatic flexura of the colon with metastases in the mesenteric glands (the puncture was performed on the glands during laparotomy), 2 cases of cancer of the stomach with metastases in the supraclavicular and cervical

glands, and 1 case of sarcomatous glands. In all the cases the diagnosis was confirmed by clinical and histological investigation.

In the cases of *carcinoma of the lungs* with metastases in the cervical glands, more or less abundant neutrocytes and scanty lymphocytes in a state of advanced degeneration were observed in lymphadenograms. From this background stand out well preserved cells with oval, spherical or polygonal shape. They are found singly or in pairs in the mass of half or completely degenerated neutrophils and lymphocytes. Usually they are not larger than leucocytes, but sometimes three or four times larger cells are seen. Their nucleus is strongly basophil and large in comparison with the cytoplasm, which is uniform, without any granules, and also stains deep basophil. Very often nucleus has just divided or is in stage of mitosis. In many of the cells nuclei as well as cytoplasm show distinct signs of degeneration (Fig. 14). The neoplastic character of these cells may be implied from their atypical structure, marked basophilia of their nuclei, and very frequent mitotic figures.

In the case of *carcinoma of flexura hepatica*, a puncture of the mesenteric gland invaded by neoplasm was performed during laparotomy. In smears single cells, one or two in each field of vision, three or four times as large as lymphocytes were seen. Their shape was round or oval, sometimes with one large bud. The cytoplasm was strongly basophil, without any structure, containing coarse very basophil granules, clearly seen only in the lighter spots of cytoplasm (Fig. 15, 16). Some of the cells appear as dark uniform bodies only, without a trace of structure, others are in a state of disintegration. The background is formed by crowded lymphocytes and lymphoblasts. The increased number of reticular and plasma cells attracts attention.

In the case of *carcinoma of the stomach* the enlarged cervical gland was punctured (diagnosed histologically in the Pathology Dept. of the Jagellonian University as carcinoma cylindrocellulare). The erythrocytes appear as in the normal lymphadenogram; the number of neutrophils is increased, in some fields some ten or more can be seen. Some of them show degenerative changes in the form of vacuoli in the cytoplasm. A few neutrophil «stab cells» were seen; there were no younger forms. A few typical lymphocytes and lymphoblasts were observed. The reticular system was represented by single monocytes similar to those found in peripheral blood, and typical reticular cells which were very numerous, some-

times outnumbering other cell elements. There was a definite increase of *Türk's cells*.

In almost every field of vision the presence of a few cells that are never found in a normal lymphadenogram, attracts attention. They lie either single or quite often in pairs, giving the impression that they are in a state of division. Sometimes a group of some thirty or more of these cells may be observed. Only those lying on the edge of such a group may be well differentiated; the rest form a packed mass, and except that the cells are all similar to each other, no definite details can be observed. The single cells (Fig. 17) measure from 15—20 to 25 μ . They have usually a slightly oval, at times an irregular shape, especially when they lie close together. The cytoplasm forms a wide boarder staining a light blue and sprinkled with very fine colourless vacuoli. In some cells, especially those lying in groups, the cytoplasm stains a strong basophil and shows much less vacuolisation. When compared to the amount of cytoplasm, the nucleus is small, 4—6 μ , strongly basophil, with fairly dense structure. Only in some nuclei there are small light spots that may be nucleoli. Usually the nuclei lie in the centre of the cell and only when the cells are found in pairs are the nuclei to be seen on the edges of the cells, opposite each other. In that case the cells and their nuclei seem to be a bit smaller, as if they had just divided. It should be noted that from time to time the cells with thick irregular loops of chromatin and binucleated cells may be observed. The above described cells, appearing in this case in large numbers, are not found in the normal lymphadenogram. They seem to us to be of neoplastic character. In histological section of the same gland, even though the cells were noticeably smaller, they had similar characteristics, in that there was a strong vacuolisation of the cytoplasm.

Sarcoma. A smear from an enlarged, hard axillary gland was made in which the histological examination in the Pathology Dept. of J. U. showed sarcoma variocellulare. *The erythrocytes* were similar to those in normal gland. *The granulocytes:* in many fields of vision there was a noticeable increase in the number of polymorph neutrophils and eosinophils. A large number of them showed degenerative changes in the form of vacuolisation and complete disintegration. In comparison with the normal lymphadenogram there were much fewer typical *lymphoblasts* and *lymphocytes*. There was a great increase in number of typical *reticular cells*; in some fields of vision they were lying side by side composing

the majority of all elements. Their cytoplasm was usually well preserved, some nuclei were stripped of cytoplasm. A large number of the cells, well above the normal showed the appearance of macrophags, with formless strongly basophil inclusions, occasionally with red cells and lymphocyte nuclei phagocytosed. *Mono-cytes* were also present in numbers that were above normal. A special notice should be taken of cells (Fig. 18) that never were seen in the normal lymphadenogram. These cells vary in size from 7—15—20 μ . They are usually round and only when several cells are found together are they subject to mutual flattening which gives them an oval or irregular outline. The whole cell stains a strong basophil. Only in some cells it is possible to differentiate a large nucleus, without nucleoli, from the deep basophil cell-body; in others the nucleus fills almost the whole cell and can hardly be distinguished from the cytoplasm. Some of the cells lying side by side give the impression of undergoing division. It is characteristic of these cells that they stain a strong basophil and for this reason they can be easily distinguished from the neighbouring forms. It is quite definite that they are neither over-stained lymphocytes nor reticular cells. Proof of this is in the fact that they are found either singly in the neighbourhood of other known, normally stained cells or that typical normally stained reticular cells, lymphocytes, and granulocytes lie singly among the numerous strongly basophil cells described above. We consider these cells to be of neoplastic origin; the histological preparation confirms this.

Concerning the use of lymphadenogram for diagnosis of the neoplastic lymphatic glands, we must state that owing to the very few cases of ours and not very numerous cases of other authors, one cannot give yet any definite morphological principles on which to base such diagnosis. However on the basis of our present material we can indicate certain characteristics that are found in the lymphadenogram of neoplastic glands. These characteristics are: The different general appearance of the neoplastic cells when compared with all cell elements of a normal lymphadenogram. The most striking is very strong basophilia both of the nucleus and cytoplasm, sometimes so pronounced that the boundaries between them are completely effaced and it is impossible to distinguish any cell structure. The cells show very often degenerative changes, principally in the form of vacuolisation of the cytoplasm. Very characteristic is the marked tendency of these cells for atyp-

ical division with formation of thick irregular chromatin loops (atypical chromosomes?) during mitosis and development of binucleated cells showing asymmetry of nuclei with regard to their size and the position within the cells. The presence of mitotic figures, especially of atypical ones, may be taken as evidence of the great proliferative power of these cells. The above described basophilia, lack of any definite differentiation and in the same time the signs of degeneration suggest also an intensified and pathological proliferation.

Conclusion.

In summing up the results of our research we can make the following statement as to the clinical value of the lymphadenogram, even though our material is too small to draw any final conclusions: The lymphadenogram in lymphadenoma (Hodgkin's disease) is of real diagnostic value only if it is, as we emphasized above, «a complete lymphadenogram». The lymphadenogram in myeloid leucemia may be of real help in the aleucemic form with myeloid metaplasia of the lymphatic glands. And finally in case of neoplastic glands lymphadenogram may at least facilitate the diagnosis and diagnostic procedure.

All the microphotographs were prepared in the Bacteriology Department of Jagellonian University (Director: Prof. Dr. M. Gieszczykiewicz †) with the help of Dr. Przybytkiewicz. We wish to thank him for his kind assistance.

References.

1. H. Fleischhacker and R. Klima: Münch. Med. Woch. Nr 17. 1937.
 - 2. B. Ginsbourg: Le Sang. 334. 1935. — 3. M. Kubiczek: Pol. Gaz. Lek. Nr. 9. 1938. — 4. F. J. Lang: Handbuch der allgemeinen Hämatologie. B. I. 2. Herausgegeben von H. Hirschfeld u. A. Hittmair. Urban & Schwarzenberg 1933. — 5. B. Petryński: Pol. Arch. Med. Wew. T. XVI. Z. 3. — 6. C. Sternberg: Ergeb. d. allg. Pathol. u. Pathol. Anat. T. XXX. J. F. Bergmann 1936. — 7. R. Stahel: Diagnostische Drüsenpunktionen. G. Thieme. Leipzig. 1939. — 8. T. Tempka and M. Kubiczek: Fol. Haemat. 60. 18—37. 1938. — 9. W. Tischendorf: Deut. Arch. f. klin. Medizin. 183. Z. 4. 448. — 10. E. Weil, I. Wall and S. Perlès: Bull. et Mém. de la Soc. Méd. d. Hôp. d. Paris. Nr. 21. 1936. — 11. The same: La Presse Médic. Nr. 80. 1936. — 12. F. Velasco Montes: Münch. med. Wschr. 7. 1939. 225—258. — 13. A. Venduvre, P. Ingelrans and Nigoul: Soc. de Méd. d. Nord. 18. III. 1937.
-

From the Department of Physiology, University of Oslo.

Studies of Erythrocyte Counting.¹

I.

Technical Errors.

By

HENRIK F. LANGE and HERBERT PALMER.

(Submitted for publication November 21, 1947.)

A. General Remarks.

In the hematological literature we find numerous papers on the counting of erythrocytes and the normal values found therefore. One should therefore suppose that the subject had been thoroughly debated.

Meanwhile it appears that under given circumstances we meet with conditions which diverge so greatly from the accepted standards that it seems necessary to take up for renewed consideration the technique employed and the value recorded.

Special investigations which the authors pursued separately in 1943 also included enumeration of erythrocyte. The material at disposal was the same in both cases, namely, healthy medical students who were then doing service at the University Physiological Institute in Oslo.

A remarkable finding was that most of the students showed erythrocyte values which were a good deal lower than the average normal figures usually noted in the Scandinavian countries.

This led to the problem being taken up for investigation by the authors in collaboration.

The low figures might be due to methods employed, to physiological peculiarities (war time phenomena) or to both factors.

¹ The work has been supported by grants from Freia Chocolate Fabriks Medicinske Fond.

The necessary requirement for the judgment of these matters was then an examination of the technical details in counting of erythrocytes. In other words, the first part of the task was to make clear the methodological errors in the widest sense and then on the basis of this scrutiny to demonstrate the most accurate method (our absolute figures have been published, with comments, in the *Acta Med. Scand.*).

The factors which influence the results of the erythrocyte counts are usually divided into two categories:

1. Methodological errors. 2. Physiological errors.

We have preferred to divide them into:

1. Technical errors.
2. Technical-physiological errors.
3. Physiological variations (less appropriately called physiological errors).

1. The technical errors comprise:

- a. *Errors due to apparatus and reagents:* Counting chambers, cover-glasses, pipettes for blood-taking and dilution, dilution fluids.
- b. *Errors of execution:* Measurement of blood and dilution fluid, mixing of blood with the dilution fluid, filling of the counting chamber, imposition of the cover-glass, counting of the erythrocytes.
- c. *Other technical errors:* Distribution of the erythrocytes in the counting chamber, evaporation of the liquid in the chamber, variations in the surrounding temperature etc.

2. *The technical-physiological errors* concern the technique employed in taking blood-samples. The basis for this terminology is the following: The damage to a small portion of the organism which the puncture in itself represents calls forth in the vessels reflex reactions of defensive and reparatory nature which will have a varying influence on the number of erythrocytes. There will here arise an interplay between the technical procedures and the organism. It is then important to endeavour to find the technical method that will call forth the least physiological reaction.

This group thus comes to embrace: Preparation of the skin, the puncture, the relation to the blood stream, the drawing up of blood into the pipette, the choice of place (localisation) for the puncture (ear, finger, vein).

3. *The physiological variations* comprise:

The posture of the subject of experiment, eventual daily rhythm, constitutional elements (race, sex, age), external factors (climate, height above sea-level) etc.

After having carefully gone through these factors, one by one, we found it necessary to concentrate our investigation upon some of them in particular. As regards many of the factors, we could, as was to be expected, merely confirm the results of earlier investigations. *These* factors will therefore not be dealt with here.

In the following there will be given an account of our investigations concerning what we regard as »weak points» in the methods, wherein we have, broadly speaking, followed the main classification suggested in the above description of the influencing factors.

In these investigations the following apparatuses and liquids have been employed: Buerker's counting chambers with rectangular cover-glass, without clamps for fixing the coverglass (except for special purposes — see later). Further, Ellermann-Jørgensen's system with separate pipettes for blood and for dilution. Mercury-calibrated pipettes (10 cmm) have been used for the blood and ordinary 2-ml full-pipettes for the dilution liquid. We found the use of 2-ml pipettes more convenient and accurate (for example, it is easier accurately to measure 2 ml than 1990 cmm). It was found that special Ellermann pipettes for the dilution liquid, with graduation for 1990 cmm volume, were very inexact, as delivered from the makers. (Adjustment of three such pipettes showed the following volumes: 2024, 2018 and 1976 cmm.) Mixing pipettes were not used at all. They are difficult to adjust exactly and are usually subject to a considerable degree of error during use.

As mixing tubes were employed ordinary dwarf test-tubes with rubber stopper.

For puncturing was used a thin, sharp »cataract knife» (for special purposes a scarificator — see later).

As dilution liquid was regularly employed 0.9 per cent NaCl solution (for special purposes Hayem's solution — see later). For disinfection of the skin ordinary technical ether was used.

A careful mode of procedure was adopted for the pipetting (the tip of the pipette was wiped after filling, the blood pipettes were rinsed with the dilution liquid, regard was paid to the drainage time).

B. Studies of the Counting Chambers.

Especially by earlier investigators it was rightly maintained that in serial counting of erythrocytes the same counting chamber and the same pipettes should always be used in order thereby to eliminate errors due to the apparatus. This requirement can naturally be upheld so long as it is a question of a particular series where the observations do not follow in too close succession, and where they are preferably made by the same investigator. This demand does not hold good when series taken from different places are being compared and when the serial observations are made at such short intervals that there is no time for cleaning the apparatuses.

In recent years (since 1930) it has constantly been asserted (for example, by P. Färgeman) that the counting chambers which are now being manufactured in one piece are so accurately constructed that any possible divergency between the different chambers (of same make and type) will be so extremely small that it can be left out of consideration. It is, of course, quite possible that these appliances possess such exactitude, but the counting chambers are made of glass and are subjected during use to various influences (varying temperature, friction etc.) which affect the physical structure of the glass. It must therefore be presumed that there may arise alterations in the counting chambers which may be of practical significance for the results of the counting.

Physical Measurement.

In order to get some idea as to whether such a divergency existed, physical measurements¹ were made of the height of the chamber in three, fortuitously chosen Buerker counting chambers of the same type (two without clamps, No. 93595 and No. 41752,

o

and one with clamps, oKa). The same cover-glass was used in all

a

cases. The measurements were made on *both outer edges* and on the *inner edge of the counting field*. Three measurements were made

¹ The physical measurements in this work were made by Amanuensis Nils Gulberg Olsen, University Physical Institute, Dept. A., Oslo.

at each place, with an exactitude of 0.001 mm. Clamps were not employed during the measurement.

The results are given in Table 1.

Table 1.

Physical Measurement of Height of Counting Chambers.

	Buerker 93595		Buerker 41725		Buerker ^o Ka a	
	Inner edge	Outer edge	Inner edge	Outer edge	Inner edge	Outer edge
Mean value	0.1085	0.1080 mm	0.1050	0.1030 mm	0.1040	0.1030 mm
Total mean value.	0.1080 mm		0.1040 mm		0.1035 mm	

The chambers show an average error in height of respectively + 8, + 4 and + 3.5 per cent, In the calculation we reckon, as is known, with a height of 0.1000 mm. Accordingly we see that none of the chambers had this height.

It may possibly be permitted to reckon with a corresponding result as regards the quadratic pattern of the counting fields.

As to whether these divergencies arose after delivery from the factory or were present already at the time of delivery no opinion can be formed without special investigation of the matter, which we had no opportunity of making.

In all cases we will already at the point of time note a varying, but absolute, error, which will be camouflaged in serial investigations with the same chambers, but which may become manifest when several chambers are used.

The practical conclusion to be drawn here from is that the counting chambers should be calibrated from time to time by physical measurement (with introduction of an eventual factor) as is done as a matter of course in case of other measuring appliances.

Meanwhile, if the physical measurement is to be fully satisfactory, it should be done at several places in the counting chamber, including the counting fields, but even on measuring relatively few, but important places, as in our case, it will be seen that the height of the chamber varies from place to place (see Table 2).

Consequently the volume per unit of surface will also vary from place to place. In order as far as possible to eliminate these

Table 2.

Physical Measurement of Height of Counting Chamber (Details).

	Buerker 93595		Buerker 41725		Buerker $\frac{o}{a}Ka$	
	I. E. ¹	O. E.	I. E.	O. E.	I. E.	O. E.
Outer	0.107 mm 0.110	0.110 mm 0.107	0.104 mm 0.105	0.103 mm 0.102	0.106 mm	0.102 mm
Middle	0.108 0.108	0.110 0.110	0.107 0.105	0.103 0.105	0.106	0.102
Outer	0.110 0.108	0.106	0.105	0.103	0.103	0.105

variations it is necessary to count a sufficient number of squares (blood corpuscles) in definite order and to make use of both counting fields.

Although by physical measurement we may get a corrective factor for the average count in the chamber, we cannot, owing to the above-mentioned variations of volume, preclude the possibility of a difference between the two fields.

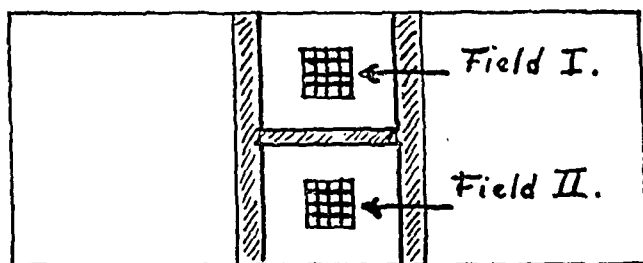


Fig. 1.

The result of an investigation embracing 255 comparisons between field I and field II (see Fig. 1), is shown in Table 3, where only the preponderance in one or the other direction is recorded.

Calculated in percentage, field I shows preponderance in numbers of erythrocytes in 58.3 % of the total countings, field II shows preponderance in 41.7 %, *i. e.* that field I shows 16.7 %

¹ I. E. Measurement made on inner edge. — O. E. Measurement made on outer edge.

more positive findings than field II. The difference between the two fields was not particularly great, but is it large enough to indicate that it is an error in procedure not to count both fields.

Table 3.

Distribution of the Number of Counted Erythrocytes in Two Fields, Denoted by + and —.

Field I	147 + (105 —)
Field II	105 + (147 —)

Calibration by Means of Erythrocyte Counts.

The question whether, instead of proceeding to physical calibration of the chambers in each individual case, we can attain our object by comparative counts of the blood cells in two chambers, of which one has been physically calibrated, is of immediate interest. In such case we could use the physically calibrated chamber as »prototype».

It has been tried to elucidate this question by comparing the counts in two of chambers already physically measured (Buerker 93595 and Buerker 41725). As will be remembered, these chambers had different heights, 0.108 and 0.104 mm respectively. The result will be seen from Table 4 (where K_1 denotes Buerker's chamber 93595 and K_2 Buerker's chamber 41725).

Table 4.

Comparison Between Chambers K_1 and K_2 . Average Figures.

Number of obs.	K_1	K_2	Diff.	Diff. given in percent.		$K_1 > K_2$	$K_1 < K_2$	$K_1 = K_2$
				Average	Dispersion			
14.....	4.42	4.22	0.20	4.6	+ 11.1 — 0.0	13	0	1

Thus we see that in 13 out of 14 cases chamber 1 has a higher number of blood cells than chamber 2. The statistical analysis shows, however, that this difference is not certainly real, but lies within the range of error of the method. (The real difference, when $\pm t \geq 2.778$, t is here 1.448.) In other words, this will

camouflage the difference between the chambers. This, again, will mean that an eventual difference between the chambers cannot be very great. Buerker maintains that differences in height of the chamber — provided that Newton's colour rings are present — are of no particular practical importance. For so far, our results may be said to confirm this statement, but that does not mean that the counting method is a suitable method for reliable calibration of a counting chamber.

Counting Chambers With and Without Clamps.

The cover-glass plays an important rôle. It must lie absolutely flat, have smooth (polished) surfaces and be sufficiently large. It must be so placed that Newton's rings appear on both cross-beams, and these rings must be of the same order, a point which experience tells us is often neglected.

There then arises the question whether we shall use chambers with or without clamps for fixing the cover-glass.

With use of clamps three situations may theoretically arise: 1) the cover-glass may curve downwards — whereby the height of the chamber becomes too small. 2) It may curve upwards — whereby the height of the chamber will become too great. 3) It may be fixed absolutely level. This last will probably most often be the case. These three situations will depend upon the type of clamps used, on the point of fixation to the chamber and, not least, upon the pressure to which the clamps are subjected.

In the first two cases it will be difficult to attain formation of Newton's rings. But it is a constantly recurring experience that it is altogether difficult to secure a proper control of the Newton's rings when using clamped chambers. It will also be found that the rings vary according to the pressure exerted on the clamps, and it will be difficult to ensure that the colour rings are of the same order on both sides of the chamber.

In case of physical measurement it was also found that by use of clamps the distance between the cover-glass and the cross-beam of the chamber can be varied within the area where Newton's rings appear by about 0.002 mm, or by practically speaking 2 per cent of the height of the chamber.

In order to get some idea of the practical importance of clamps, 28 parallel counts were made in chambers with and without clamps. The chambers used for this purpose — and in all sub-

sequent investigations in this work — were corrected in order to attain comparable results.

The result of this comparison is given in Table 5 (where K_m denotes chamber with clamps and K_u chamber without clamps).

Table 5.

Use of Chambers With and Without Clamps. Average Figures.

Num- ber of obs.	No. of E. in chambers with clamps	No. of E. in ch. without clamps	Diff.	Diff. in percent.		$K_m > K_u$	$K_m < K_u$	$K_m = K_u$
				Average	Dispersion			
28....	4.38	4.13	0.25	6.47	+ 18.45 — 5.4	25	3	0

In 25 out of 28 cases the clamped chamber shows a higher number of erythrocytes. Meanwhile the statistical analysis shows no certain real difference. (There is real difference when $t \geq 0.387$, while t is here 0.25.) But nevertheless we get the impression that there exists a difference that comes very near to the real difference.

This finding, when compared with the physical measurement and the theoretical possibilities of error, as mentioned above, renders it advisable to refrain from the use of clamped chambers.

By using chambers without clamps we can avoid convex positions of the cover-glass and we have greater chance of always obtaining Newton's rings of the same dimensional order, since we will here have to do only with adhesive forces.

Filling of the Counting Chamber.

In the filling of the chamber there is one »error» which is very common and very easy to do, namely, overfilling. A method generally employed in most hospital laboratories, as prescribed in the ordinary textbooks on laboratorial technique, is immediately to suck up the supernatant liquid by means of filterpaper. We find little mention in the literature of the influence hereof on the results of the counting.

In Table 6 are seen the results of 27 experiments with comparison of exactly filled chambers (»autofilled») — and overfilled chambers from which the excess liquid was absorbed.

Table 6.

Average Figures.

No. of obs.	Ex- actly filled	Over- filled	Diff.	Diff. in percentage		Exactly filled > overfilled	Exactly filled < overfilled	Exactly filled = overfilled
				Aver.	Dispersion			
27....	4.25	4.19	0.06	1.22	+ 11.9--15.3	16	9	2

No difference can be noted. (The numerical difference of 1.22 per cent is not statistically tenable. The real difference is $t \geq 0.25$, t is here = 0.06.)

It must, however, be emphasized that the absorption of the excess liquid ought to be effected immediately and with caution.

Error Due to the Distribution of the Blood Cells.

One of the most constantly arising errors in the counting of blood corpuscles is that due to their *uneven distribution* in the chamber. The cells are always found to be unequally distributed. The cause of this inequality is at present unknown, in spite of many attempts to explain it.

The question whether the sedimentation rate and adhesion between the cells have any influence as regards this source of error has been investigated by a number of authors. Both these phenomena are dependent on the concentration of the cells and are therefore not the same for white and for red corpuscles. Meanwhile, the distributional inequality is independent of the cellular concentration and is the same for both kinds of cells, also when diluted in their respective dilution fluids. Attempts have been made to produce dilution fluids with the same specific gravity as the erythrocytes by adding different substances, or by reducing the sublimate concentration in Hayem's solution, in order thereby to diminish or eliminate the sedimentation rate. This procedure has likewise had no influence on the inequality of distribution.

The mean sedimentation rate for erythrocytes in Hayem's solution is, according to Behrens, about 0.1 mm per minute. The filling of the counting chamber can hardly take more than from 1 to 3 seconds. It is therefore difficult to imagine that the sedimentation rate during the process of filling would have any effect as an »de-miscation factor».

A modification of the diluting liquid is therefore not likely to lead to a more uniform distribution.

The question whether the time taken in mixing the solution beforehand has any influence on the inequality of distribution has been investigated (Buerker). From this investigation it appears that the unevenness of distribution is the same, whether the time taken for mixing the dilution liquid is 10, 3, 2, or 1 min. Buerker therefore recommends mixing for 1 minute before filling the chamber. In our experiments we have mixed the solution about 100 times (cautiously, without shaking), which corresponds to 1 minute and 40 seconds.

In the foregoing we have used only the expression »unequal distribution in the chamber», and from the literature it does not seem clear whether the localisation of this uneven distribution is *systematic* or *fortuitous*.

Our experiments, however, seem to show that there is a typical system in the unequal distribution. It was remarkable to note how often we found more blood cells per square in the inner part of the chamber.

The meaning of the designations: inner, outer and central will be seen from the following diagram:

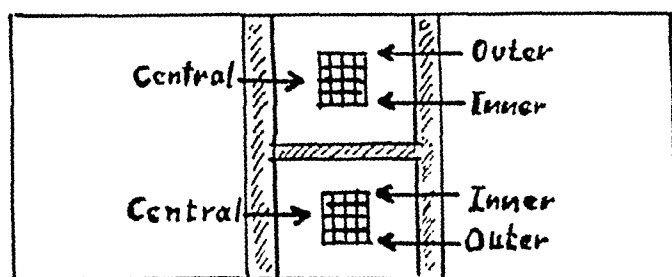


Fig. 2.

Investigation a) embraces 38 parallel counts in blood in the same dilution. There were counted altogether 16 »CD» squares (abt. $1/800$ mm) in each field, respectively inner and outer. The results are given in Table 7.

There is a distinct difference in the results of the counts, with a higher number of erythrocytes in the inner part of the chamber.

Statistical analysis shows a real difference of 16.5 per cent (real when $t \geq 0.085$. t is here 0.39).

Table 7.
Average Figures.

No. of obs.	Inner	Outer	Diff.	Diff. in percentage		Inner > outer	Inner < outer	Inner = outer
				Average	Dispersion			
38....	2.30	1.91	0.39	16.5	+ 31.5- -0.5	38	0	0

Investigation b), in which the mode of procedure has been the same as in a), embraces 100 parallel counts with different blood-samples.

The results are given in Table 8.

Table 8.
Average Figures.

No. of obs.	Inner	Outer	Diff.	Diff. in percentage		Inner > outer	Inner < outer	Inner = outer
				Average	Dispersion			
100....	4.48	4.26	0.22	4.76	+20.3- -13.0	81	19	0

Likewise here it is distinctly seen that the concentration of erythrocytes is greatest in the inner part of the chamber (81 out of 100 cases). The difference in the results of the counts, however, is not so great as in *Investigation a)*, but it is real and amounts to 4.76 per cent. (Real difference when $t \geq 0.17$, t is here 0.22).

In order to get an impression of the distribution in the central part of the chamber 5 parallel counts, in the centre and outer part, were made in *Investigation c)*.

The results are set forth in Table 9.

Table 9.
Average Figures.

No. of obs.	Cen- tral	Outer	Diff.	Diff. in percentage		Central > outer	Central < outer	Central = outer
				Average	Dispersion			
5.....	4.30	4.15	0.16	3.5	+ 9.2- -2.6	4	1	0

No real difference can here be noted. (The number of observations however is too small to admit of a certain judgement.)

From these investigations it may be concluded that there takes place a systematic concentration of the blood cells (erythrocytes) in the inner half of the counting fields. It can be ascertained (visually) that this increase in concentration is greatest at the inner peripheral edge of the fields. The same phenomenon has been observed on use of several counting chambers. The cause of this systematic distribution has not been made clear.

The practical conclusion to be drawn from these investigations is that we must endeavour to adopt a systematic method of counting. Taking everything into consideration, *we have found it most expedient to count diagonally*. If, for example, we count 16 CD-squares in each field, we can in this manner count 12 CD-squares diagonally, and of the remaining 4 CD-squares we then ought to select one in the inner, one in the outer and two in the central parts of the fields.

C. Studies of the Dilution Liquids.

Since the latter part of the last century Hayem's solution has maintained its position as an excellent medium for dilution for erythrocytes. Meanwhile, we found it simpler (as regards the technique of preparation) to employ a 0.9 per cent NaCl solution for this purpose. The object of the following investigation was to compare these two dilution liquids in order to get an idea of the suitability of the 0.9 per cent NaCl solution.

In *Investigation a)* parallel counts were made immediately after dilution of the blood with 0.9 per cent NaCl solution and with Hayem's solution respectively. Five different blood-samples were employed.

The results are shown in Table 10.

Table 10.

No. of obs.	In NaCl solution	In Hayem's solution	Diff.	Diff. in percentage		NaCl > Hayem	NaCl < Hayem	NaCl = Hayem
				Average	Dispersion			
5.....	4.26	4.25	0.01	0.2	+ 4.8- -4.9	3	1	1

There was here found no difference in number of erythrocytes on use of the two solutions.

Investigation b) concerned the behaviour of the erythrocytes after standing for some time in both solutions.

During the first 8 hours the results of the counting remained the same and unchanged for both suspensions. Afterwards there came a slight gradual fall in both cases.

After 24 hours the average decrease was 5.0 per cent for the NaCl solution and 4.4 per cent in Hayem's solution.

Thus there was no particular difference between the two dilution liquids, and it was found fully admissible to use a 0.9 per cent NaCl solution as diluent, at any rate in our investigations, where the counts were generally made immediately after dilution.

It must, however, be added that the strength of the NaCl solution shall be exactly 0.9 per cent, since 0.8 per cent or 1 per cent solutions give varying and sometimes greatly divergent results.

It is also necessary that the NaCl solution should not be too old. After a longer or shorter time there regularly comes microbic pollution. The NaCl solution is then no longer usable and must be changed.

Summary.

An account is given of some investigations respecting certain, purely technical, sources of error in the counting of erythrocytes, which have hitherto received little attention.

1. On physical measurement of the height of three Buerker counting chambers there were found variations in height, in average + 8, + 4 and + 3.5 per cent.

Physical calibration of the counting chambers from time to time is recommended, or at any rate physical control examination.

2. Variations in the height of the chambers may occur through use of clamps for fixing the cover-glass.

By use of chambers without clamps it is easier to avoid variations in height and easier to obtain Newton's colour rings of the same order.

3. Absorption of excess liquid from »overfilled» chambers has no influence on the results of the counting.

4. It is shown that the unequal distribution of erythrocytes in the counting fields is *systematic*, with increased concentration in the inner half of the field.

The cause of this phenomenon, as well as of the distribution anomaly, is not clear, but it implies that the counting should

be effected in systematic order. »Diagonal» counting is recommended.

5. A 0.9 per cent NaCl solution is fully equivalent to Hayem's solution for counting within 8 hours after the mixing of the blood with the diluent. After 24 hours the number of erythrocytes shows a decrease of 5 per cent with use of 0.9 per cent NaCl solution and 4.4 per cent when Hayem's solution is employed.

Bibliography.

Behrens, F.: Pflügers Arch. 1922: 195: 266. — Færgeman, P.: Ugeskr. f. Læger, 1937: 99: 914. — Lange, H. & Palmer, H.: Acta Med. Scand. 1947: 127: 1.

From the Medical Clinic, Academ. Hospital, Upsala, Sweden.
(Acting Head: Assistant Professor J. Waldenström, M. D.)

Family Epidemic of Primary Atypical Pneumonia.

By

OLLE HOGEMAN.

(Submitted for publication November 20, 1947.)

During the last ten years an atypical form of pneumonia has been described in medical literature under such various names as acute pneumonitis, interstitial pulmonitis, acute interstitial pneumonia, disseminated focal pneumonia, atypical bronchopneumonia and other more or less descriptive titles (1—8). This form of pneumonia received particular attention during the recent war because of its great frequency in allied military camps, particularly in the U. S. A., but it has also been observed elsewhere. Since 1942 this disease has been called »Primary Atypical Pneumonia, Etiology unknown» as proposed by the U. S. Army Commission on Pneumonia (9). During the last two years this form of pneumonia has also attracted great attention in Sweden, where the disease has manifested itself both endemically and epidemically (10—11). The following is a short report of a family epidemic of this disease, preceded by a few notes on clinical features and epidemiology of the disease.

As regards distribution, the disease seems to occur over large areas of the world. In the U. S. A. it increased greatly during the war years and it has been calculated that as much as 70 % of all cases of pneumonia were to be ascribed to this group (12). The first Swedish communication concerning this disease was published in 1945 and thereafter cases were diagnosed generally throughout the country during the latter half of 1945 and in 1946,

whereas in the spring of 1947 it seems to have been rare. The reason for this increase in incidence at the turn of the year 1945—1946 and the period immediately following is obscure, it is possible, however, that there may have been some connection with a widespread influenza epidemic which occurred at that time.

The etiology of the disease is still completely unknown in spite of the great efforts which have been made to demonstrate a causal agent. The common pneumonia bacteria are absent and since the illness shows certain clinical characteristics which point to its being a virus disease, efforts have been made to isolate the suspected virus (13—15). Certain known viruses may cause pneumonia of a type which clinically is indistinguishable from atypical pneumonia. This is particularly true of influenza A & B, the psittacosis- or ornithosis virus, the virus of lymphogranuloma venereum, the meningo-pneumonitis virus, as well as special Rickettsiae and fungi, and attempts were made to demonstrate and cultivate these in long series, but on the whole with negative results (16—20).

For the greater proportion of atypical pneumonia cases none of the mentioned agents can be held etiologically responsible, nor can the indifferent streptococcus (Rockefeller strain No. 344) which has been successfully isolated from some patients suffering from atypical pneumonia (21). Intensive research was carried out by the U. S. Commission on Acute Respiratory Diseases (22) to establish the cause of the infection: healthy volunteers were inoculated with the sputa and throat washings from patients with atypical pneumonia. Three different inoculates were used: 1) untreated, 2) Seitz filtrated 3) auto-claved. The inoculate was administered by spraying in synchronization with deep inhalation. Results showed that administration of untreated and filtered inoculate produced infection with atypical pneumonia in three of the volunteers out of each group (there were 12 in each group), while on the other hand all those treated with auto-claved inoculate remained healthy (18 individuals). In the first two groups minor respiratory ailments of an undifferentiated type preceded atypical pneumonia in nearly 40 %, while among those treated with auto-claved material merely one showed such symptoms. From these investigations it has been concluded that the agent is a filtrable virus which is, however, destroyed by autoclavation.

The disease occurs both endemically and epidemically and especially attacks young people and children. It is probably

spread by aerial infection but there are indications that infection by direct contact from person to person also occurs (11). Obviously not all infected persons show the clinical picture of atypical pneumonia, many merely exhibit light catarrhal symptoms and they can thus, in their turn, spread the infection. Exposure to damp and cold is regarded by Dingle (22) as a predisposing factor in about 50 %. There are indications that other factors such as foodstuffs, milk, water, and insects are also responsible for spreading the disease (23).

The clinical picture of atypical pneumonia differs in several respects from that of pneumococcus pneumonia. The disease is usually not very acute but most often passes off after a few days with symptoms concentrated in the upper respiratory tract. Physical signs of pneumonia are unusual and are often in marked contrast to the radiographical changes which have the character of a consolidation radiating from the hilar region. A strong effect on the general condition is less usual than in the case of pneumococcus pneumonia, pleuritic pain is likewise absent (central pneumonia), cough is non-productive or productive, and even staining of the sputum is common, and persistent coughing may in fact often be the patient's greatest subjective complaint. This explains also why the most common complication in this form of pneumonia, according to Middleton, are fractures of the ribs (cough fractures) (24). Other complications are rare. Temperatures vary, they may be very high and often persist for a long time up to several weeks and then fall as a rule by lysis. Among the more important laboratory tests it may be stated that leucocytosis is not usually present; a value of over 10,000 was found in only 1/3 of the cases. The sedimentation rate is as a rule high. As a further difference from the ordinary pneumococcus pneumonia we should note the resistance to specific chemotherapy, such as the sulphonamide group and penicillin.

In the diagnosis of atypical pneumonia the cold-agglutination described by Turner and his collaborators has played an important part (25). Here in Sweden Löfström, Laurell, and Hedlund (10) were the first to draw attention to this reaction, but as they point out in their publication this is not a necessary phenomenon. In various American reports figures are given as varying between 30—70 %. The fact seems to be that the higher a patient's temperature or the longer it lasts, the greater is the chance of a positive agglutination in the later period of the illness. A value above

1/8 must be regarded as pathological; but more important than a single pathological value is the mounting titration value from the first to the second week. It is very important that the person performing these tests should take all technical precautions and *inter alia* use fresh O-erythrocytes, small amounts of erythrocytes etc.

The epidemic which will be described below happened on a large estate some kilometres from Stockholm. The family consisted of the owner and his wife, a married son, four unmarried daughters and an old housekeeper. All the members of the household became ill with the exception of the father and the old housekeeper; on the other hand, although the wife fell ill, the symptoms were much milder in her case than in the case of the others. It started with the return of the son (1. J. O. C.) who arrived home at the end of July from a visit to Denmark, where he had studied agricultural methods for a few weeks. About July 20th he had paid a visit of a few days to a large poultry farm where all kinds of birds were kept. He could not say that he had intimate contact with these animals, but he had thoroughly studied arrangements for their feeding, housing, fencing-in etc. For the rest of his stay in Denmark he can not remember having been in contact with anybody who was ill or »had a cold», nor had he heard of any epidemic of colds at that time. He arrived home in Sweden on August 1st and fell ill on the 6th with a feeling of general lassitude, coughing and fever. He was treated at home for about a week, confined to bed and given sulphonamides because of suspected pneumonia but there were no physical indications for this diagnosis. As however in spite of this treatment the temperature still rose and the general condition of the patient deteriorated, he was admitted to the Löwenströmska Lasarett on August 12th. During his stay at home he was nursed by his wife (2. B. C.), who fell ill with the same symptoms as her husband on August 21st. On August 23rd a sister (9. Ch. C.) and the mother (3. B. C.) became ill in the same manner, though the mother, as has already been mentioned, only very slightly.

The day after the brother fell ill, the three younger sisters left home and returned only some days after he had been admitted to hospital. About a fortnight after the mother (3) and the elder sister (9) were taken ill, the other sisters also succumbed in the following order: one on September 7th (5. B. I. C.), one on September 11th (4. A. C. C.), and one on September 15th (6. I. C.).

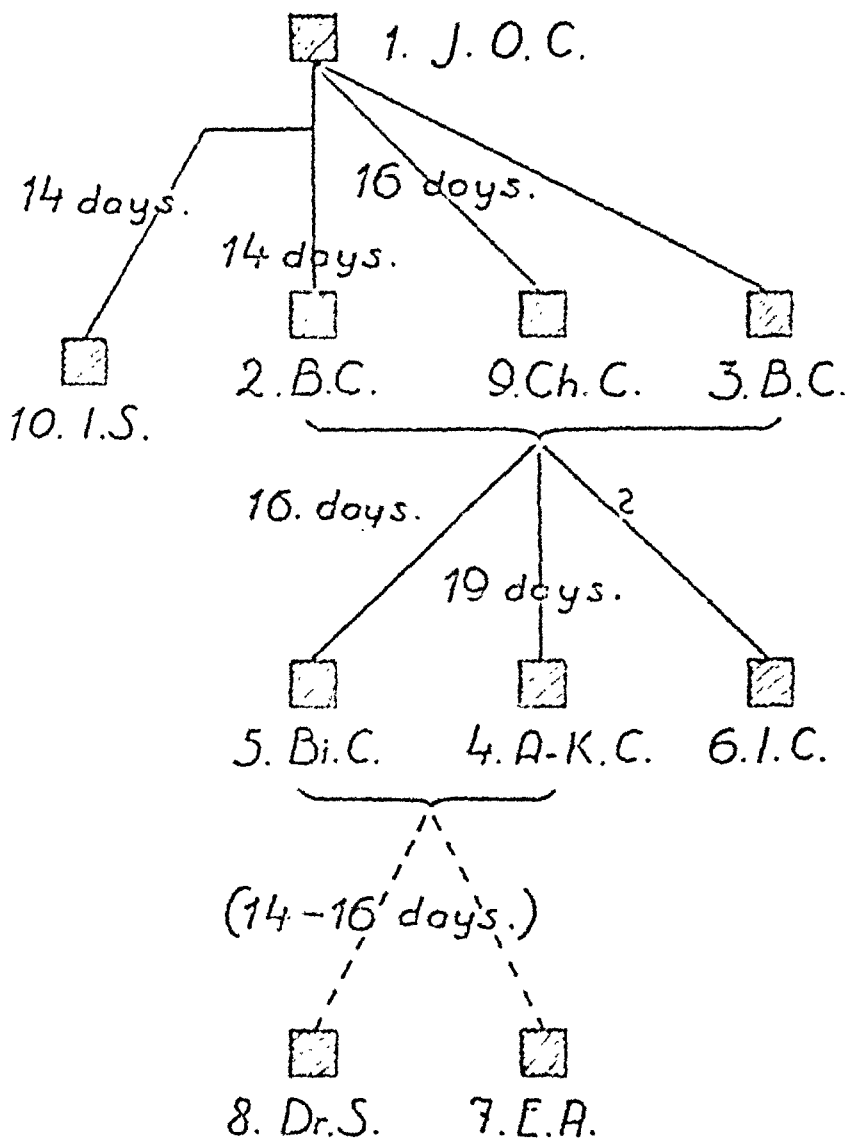
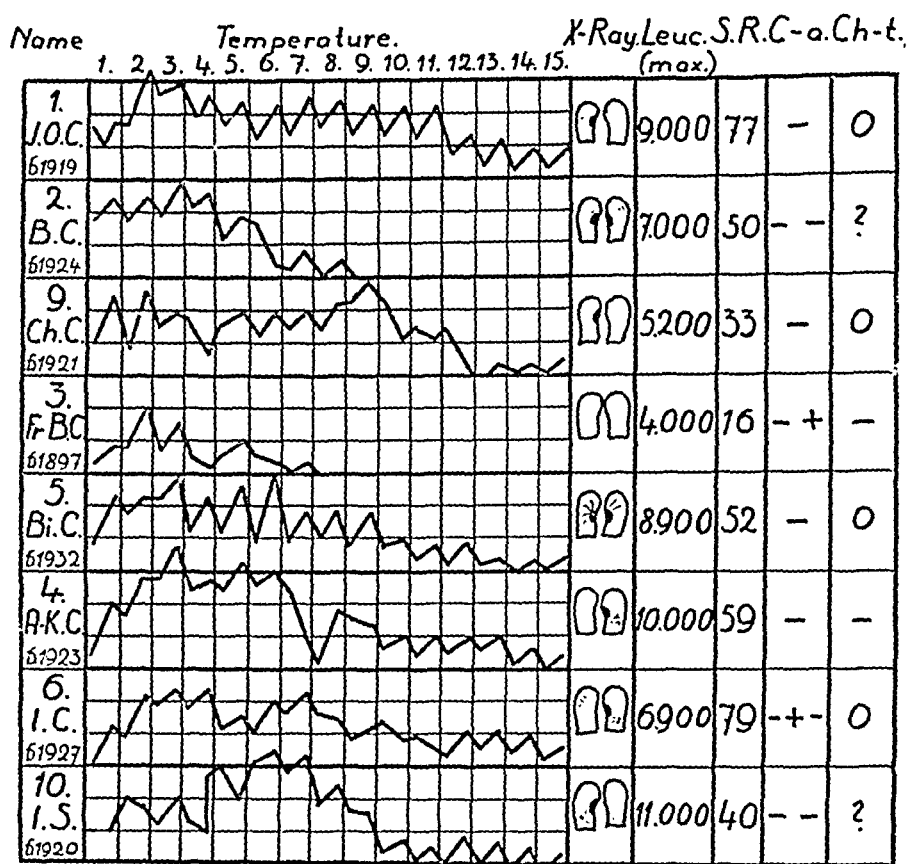


Fig. 1.

Exactly 14 days after the son's (1. J. O. C.) admission to the Löwenströmska Lasarett, the nurse (10. I. S.) attending him fell ill. In this hospital where at the time the state of health of the staff had been very good, some weeks later a nurse (7. E. A.) and a doctor (8. Dr. S.) fell ill with catarrhal symptoms, fever up to 40° lasting for several days, and severe coughing. No X-ray examination of the lungs of these two patients was carried out.

Tab. I.



S.R.: Sedimentation rate.

C-a.: Coldagglutination (over $\frac{1}{8}$).

Ch-t.: Chemotherapy (effect).

The disease ran in the main the same course in every case. Tab. I shows the temperature curves with the results of X-ray examinations and the most important laboratory tests. Only in one case (10. I. S.) were the leucocytes higher than 10,000 (only the maximum value of several tests is given in the table), in all other cases the number of leucocytes was normal or only slightly increased in spite of continued and high temperatures. Incidentally, except for the mother, all patients showed a high sedimentation rate. None of the patients was particularly strongly affected, but a common feature of all cases was the strong irritative cough which in some cases was extremely difficult to control.

This minor epidemic in many respects recalls the inoculation experiments which were discussed by way of introduction. Of the 10 persons who fell ill 7 had distinct radiographical signs of pneumonia (in two cases no X-ray examination was made), little or no leucocytosis, a high blood sedimentation rate and an incubation period of about 14 days. In the cases where chemotherapy had been resorted to, this remained without apparent effect. Tests for cold-agglutinin were carried out on all patients except the mother (3. B. C.), but this test for agglutinin reaction was made in the early stages of the disease. Only in four cases was it carried out after 8—10 days and in 2 of these it was positive (over 1/16).

The fact of the first patient's close contact with poultry, the highly infectious nature, the low percentage of positive cold-agglutinins and an incubation period of about 14 days suggested the possibility of its being a psittacosis infection. But two factors told against this diagnosis: 1) in cases of psittacosis pneumonia the mortality is reported to be very high — 20—50 % — and even in those cases which recover the illness takes a far more violent course and is more protracted than in the present epidemic where the prognosis was very favourable, 2) the test for cold-agglutinin in psittacosis is usually negative. As, however, Dr. Frank Horsfall was visiting Sweden at the time of the epidemic, he was consulted and he kindly consented to take sera from our patients with him to America for further analysis. Sera were therefore procured from patients 1—8, from which number only 5 and 6 were still ill, the rest were in a more or less advanced stage of convalescence. The result of the serological tests executed by Dr. Harald Ginsberg in the Rockefeller Institute in New York are reported separately in the following paper (26).

The epidemic shows very clearly that patients with atypical pneumonia must be regarded as highly infectious and should be isolated. This is particularly so where there are grounds for assuming, as in the present case, that the factors for immunity are similar both for the patient and his environment. A further lesson is that the diagnosis of atypical pneumonia must not be based on the outcome of the tests for cold-agglutinin alone, but in this case, as in general, it is the clinical picture, the course of the disease together with exhaustive serological tests which should be decisive.

Summary.

After a short recapitulation of the etiology, epidemiology, and clinical picture of primary atypical pneumonia an isolated family epidemic is described in which all members of the family except two elderly persons succumbed. In the hospital where the patients were cared for, a nurse contracted the disease and a second nurse and a doctor fell ill with minor respiratory illness of undifferentiated type. On the grounds of certain peculiar features of this epidemic the possibility of psittacosis is discussed (see following paper). Isolation of the patients is recommended as well as exhaustive serological tests before diagnosis is made.

Bibliography.

1. Allen, W. H.: Acute pneumonitis. *Am. Int. Med.* 1936, 10: 441. —
2. Bowen, A.: Acute influenza-pneumonitis. *Amer. Journ. Roentgenol.* 1935, 34: 168. — 3. Daniels, W. B.: Bronchopneumonia of unknown etiology in a girl's school. *Amer. Journ. Med. Sci.* 1942, 203: 263. —
4. Fred, H.: Atypical pneumonias. *N. Y. State Journ. Med.* 1941, 41: 34. — 5. Kneeloud, Y. and Smetana, H. F.: Current bronchopneumonia of unusual character and undetermined etiology. *Bull. Johns Hopkins Hosp.* 1940, 67: 229. — 6. Longcope, W. I.: Bronchopneumonia of unknown etiology (variety X). *Bull. Johns Hopkins Hosp.* 1940, 67: 268. — 7. Scadding, J. G.: Disseminated focal pneumonia. *Britt. Med. Journ.* 1937, 2: 956. — 8. Smiley, D. F., Shorvacre, E. D., Lee, W. F. and Ferris, A. W.: Acute interstitial pneumonitis. *Journ. Amer. Med. Ass.* 1939, 112: 1901. — 9. Primary Atypical Pneumonia, Etiology unknown. *War Med.* 1942, 2: 330. — 10. Hedlund, P., Laurell, G. and Löfström, G.: Akut primär atypisk pneumoni av okänd etiologi. En ny klinisk diagnos. *Svenska Läkartidningen* 1945, 1378. —
11. Jonsson, B.: Hopade fall av virus-pneumoni hos barn. *Svenska Läkartidningen* 1946, 340. — 12. Brown, J. W.: *Proc. Roy. Soc. Med.* 1943, 36: 385. — 13. Baker, J. A.: A Virus Obtained from a Pneumonia of Cats and Its possible Relation to the Cause of Atypical Pneumonia in Man. *Science* 1942, 96: 475. — 14. Eaton, M. D., Meikeljohn, G., Van Herick, and Talbot, J. C.: An Infectious Agent from Cases of Atypical Pneumonia Apparently Transmissible to Cotton Rats. *Science* 1942, 96: 518. — 15. Horsfall, F. L., Jr., Curnen, E. C., Mirick, G. S., Thomas, L., and Ziegler, J. E., Jr.: A Virus Recovered from Patients with Primary Atypical Pneumonia. *Science* 1943, 97: 289. — 16. Finland, M., and Dingle, J. H.: Virus Pneumonias. *New England J. Med.* 1942, 227: 342. — 17. Eaton, M. D., Beck, M. D., and Pearson, H. E.: A Virus from Cases of Atypical Pneumonia: Relation to the Viruses

of Meningopneumonitis and Psittacosis. *J. Exper. Med.*, 1941 73: 641. — 18. Levine, S., Holder, E. C., and Bullock, J. G. M.: Complement Fixation for Lymphogranuloma Venereum and for Psittacosis with Frei Reactions among Pneumonia Patients. *J. Immunol.* 1943, 45: 183. — 19. Shaffer, M. F., Rake, G. and Grase, A. W., Yolk, S.: Sex Antigens in the Diagnosis and Epidemiology of Lymphogranuloma Venereum. *Am. J. Syph. Gonorr. and Ven. Dis.* 1942, 26: 271. — 20. Smadel, J. E., Green, R. H., Paltauf, R. M., and Gorgeles, T. A.: Lymphocytic Choriomeningitis. *Proc. Soc. Exper. Biol. and Med.* 1942, 49: 683. — 21. Thomas, L. et al.: Serological reactions with an indifferent streptococcus in primary atypical pneumonia, *Science* 1943, 98: 566. — 22. Dingle, J. H.: *Bull. N. Y. Acad. Med.* 1945, 21: 235. — 23. Dingle, J. H. et al.: *Amer. J. Hygiene* 1944, 39: 67, 197 and 269. — 24. Middleton, W. S.: *Med. Ann.* 1945, 247. — 25. Turner, J. C. et al.: *Lancet* 1943, 1: 765. — 26. Ginsberg, H. S.: *Acta Med. Scand.* 1948: 131, 475. Serological Studies on a Family Epidemic of Primary Atypical Pneumonia.

From the Hospital of the Rockefeller Institute for Medical
Research, New York.

Serological Studies on a Family Epidemic of Primary Atypical Pneumonia.

By

HAROLD S. GINSBERG, M. D.

(Submitted for publication November 20, 1947.)

In the preceding paper (1) the clinical picture of a small family epidemic of pneumonia of a somewhat unusual form is described. Among the recognized disease entities which may be responsible for such a localized outbreak are: 1) primary atypical pneumonia; 2) influenza A or B; 3) psittacosis (or ornithosis); 4) Q fever (or *Rickettsia burneti* pneumonia); and 5) lymphocytic choriomeningitis. It is the purpose of this paper to describe the serological studies which were carried out with sera from these patients in an effort to establish an etiological diagnosis. It will be shown that the results obtained indicate that the disease was primary atypical pneumonia.

Materials and Methods.

Serum. Serum specimens obtained from 8 patients in Sweden were brought to this laboratory by air plane. The sera were made available through the courtesy of Dr. Jan Waldenström, Upsala, Sweden. The sera were stored without preservative at 4° C.

Cold hemagglutination. Serial twofold dilutions of unheated serum, ranging from 1 : 10 to 1 : 1280 in 0.85 per cent NaCl, were prepared. To 0.4 ml of each serum dilution an equal quantity of a freshly prepared 1 per cent suspension of human group 0 red

blood cells was added. The tubes were allowed to remain in the refrigerator at 4° C. for 16—18 hours. The results were read in the cold, and the titer was taken as the final dilution of serum in which definite clumps of agglutinated red blood cells could still be observed after gentle shaking (2, 3). The racks were then allowed to remain at room temperature for 2 hours or in an incubator at 37.5° C. for 30 minutes to observe the disappearance of the agglutination. It must be emphasized that sera for this test should be collected by allowing blood to clot and separate at room temperature. If the clotting of the blood is allowed to take place at icebox temperature, the cold hemagglutinins will be adsorbed onto the red blood cells and thus will be removed from the serum.

Streptococcus MG agglutination. The agglutination tests with streptococcus MG were performed as described by Thomas et al. (4). Bacterial suspensions were prepared from 18-hour broth cultures of the microorganism. The bacterial cells were washed 3 times in 0.85 per cent NaCl, suspended in sufficient saline to give a turbidity approximating No. 5 in the McFarland scale, and killed by heating at 65° C. for 1 hour. Merthiolate in a final concentration of 1 : 10,000 was added as a preservative. Serial two-fold dilutions of unheated sera were made, ranging from 1 : 5 to 1 : 640. To 0.4 ml of each serum dilution was added an equal volume of the streptococcal suspension. The tubes were allowed to remain at room temperature for 16—18 hours. The agglutination titer was taken as the highest final dilution of serum in which there could be observed by the unaided eye definite bacterial clumps when the tubes were shaken.

Hemagglutination-inhibition with influenza viruses. Antibodies for influenza viruses were determined by the agglutination-inhibition technique (5). The PR8 strain of influenza A virus and the Lee strain of influenza B virus were used. One or the other virus was inoculated into the allantoic sac of 11-day chick embryos and, after incubation at 38° C. for 48 hours, the allantoic fluid was removed and its hemagglutination titer determined (6). Serial twofold dilutions of serum which had been heated at 56° C. for 30 minutes were made, and to 0.2 ml of each serum dilution was added an equal volume of infected allantoic fluid diluted sufficiently to yield a final concentration equivalent to 8 agglutinating units of virus. To each tube was then added 0.4 ml of a fresh 1.5 per cent suspension of chicken red blood cells. The

tubes were allowed to remain at room temperature for 1 hour, and the titer was read as the final dilution of serum in which no definite hemagglutination could be demonstrated.

Complement fixation tests. The technique for the complement fixation tests was the same in each instance, the only variable being in the preparation of the antigen used. The source of complement was a pool of serum from at least 6 normal guinea pigs, which was kept frozen at -70°C . in the CO_2 ice chest between tests. Serial twofold dilutions of inactivated sera 0.25 ml volume were made, and to each was added 2 full units of complement diluted in 0.5 ml of saline containing MgCl_2 , 0.15 mg per ml (7) and, finally, 0.25 ml of the desired antigen. The tubes were then placed in a 37°C . water bath for 30 minutes, following which there was added 0.25 ml of anti-sheep red blood cell hemolysin diluted 1:1000, *i. e.*, 2.5 hemolytic units, and 0.25 ml of a 3 per cent suspension of fresh sheep red blood cells. The tubes were again placed in the 37°C . water bath for 30 minutes. The titer was read as the final dilution of serum in which no hemolysis occurred.

a) *Psittacosis virus*. The 6BC strain of psittacosis virus, adapted to the allantoic sac of the chick embryo in this laboratory, was used for the preparation of antigen for the complement fixation test. Undiluted allantoic fluid containing psittacosis virus was inoculated in 0.2 ml quantity into the allantoic sac of 10 or 11 day chick embryos through successive passages until an infectivity titer of at least 10^{-6} was obtained. The virus was titered for infectivity by means of intracerebral inoculation of Swiss mice with 0.03 ml of each dilution. Elementary bodies could be demonstrated in the allantoic fluid of each egg passage as well as in the brains of infected mice. Pooled allantoic fluid was centrifuged in an electrically driven high speed angle centrifuge (8) at 13,000 R. P. M. for 30 minutes. The supernatant fluid was discarded. The sediment was resuspended to one-half the original volume in 0.85 per cent saline at pH 7.1 and placed in an Arnold sterilizer at a temperature of 100°C . for 30 minutes. This antigen preparation was stored at 4°C . until required.

b) *Lymphocytic choriomeningitis virus*. The W. E. strain of lymphocytic choriomeningitis virus was used in the preparation of antigen. A 10 per cent suspension in broth of an infected guinea pig brain was inoculated into 2 guinea pigs, 0.1 ml intracerebrally, 1.0 ml intraperitoneally, and 1.0 ml subcutaneously. The pigs were killed at the height of their illness on the 6th day, and a 10

per cent suspension of infected brain and spleen passed into 3 guinea pigs by the same routes and in the same dosages. They were likewise killed on the 6th day and the antigen was prepared from the infected spleens as described by Smadel, Baird and Wall (9). A 10 per cent suspension was made in physiological saline solution containing 2 per cent normal guinea pig serum previously heated at 56° C. for 30 minutes. Coarse particles were removed by centrifuging at 2,500 R. P. M. for 5 minutes. The supernate was further centrifuged at 15,000 R. P. M. for 90 minutes in the high speed concentration centrifuge. The clear claret-coloured supernatant fluid was carefully separated from the floating lipid material, and then passed through a Seitz filter previously prepared with physiological saline solution containing 2 per cent inactivated normal guinea pig serum. The antigen was then stored at 4° C. for at least 14 days before being used.

c) *Q Fever Rickettsiae*. A purified ether-extracted suspension of rickettsiae, prepared from chick embryo yolk sacs infected with the Henzerling strain of Q fever organism was used. This antigen was prepared (10) and kindly supplied by Dr. Joseph E. Smadel of the Division of Virus and Rickettsial Diseases, Army Medical School, Washington, D. C.

Experimental Results.

Each of the serum specimens was tested by the several serological procedures described above. The results of all the tests are presented in Table I. It is readily seen that the only significant diagnostic results were obtained in the tests for agglutinins against streptococcus MG and for cold hemagglutinins. In cases 1 and 6 there was demonstrated a rise in the titer of agglutinins for streptococcus MG in the convalescent sera. Similarly, in case 6 there was demonstrated an increase in the titer of cold hemagglutinins in the serum obtained on the 18th day of illness as compared to that obtained on the 3rd day of the disease. In cases 4 and 5 there was observed a significantly high titer of cold hemagglutinins in sera obtained on the 44th and 26th days after onset, respectively. With the convalescent serum of case 5 there was also a significantly high titer of streptococcus MG agglutinins. It is unfortunate that sera obtained during the acute phase of the disease were not available from each of the patients.

The results of the hemagglutination-inhibition tests with in-

ruses and rickettsiae and differentiating them from the syndrome called primary atypical pneumonia, that the laboratory procedures described have proven useful to the clinician, the epidemiologist, and the virologist.

The type specific non-hemolytic streptococcus, designated streptococcus MG, was initially isolated in this laboratory from the lungs of a fatal case of primary atypical pneumonia. Subsequently it was demonstrated in the lungs of 5 other fatal cases as well as in the sputum and throat swabs of many patients with this disease (13). Agglutinins against this microorganism, in a titer of 1 : 20 or higher, are present during convalescence in serum from approximately 50 per cent of patients with this disease (13—17). Such antibodies are found only rarely in acute phase sera or in sera from patients ill with other infectious diseases.

Agglutinins develop not only against the encapsulated bacterium as already described, but against non-encapsulated variants as well (13, 18). By other immunologic technics there can likewise be demonstrated precipitins against the type specific capsular polysaccharide, capsular swelling of the microorganism with patients' sera, and positive skin reactions on intradermal injection of the capsular polysaccharide into convalescent patients. There is not only a positive correlation between the frequency with which antibodies against streptococcus MG develop and the severity of the illness, but there is also a similar relationship between the duration of the disease and the height of the antibody titer (13, 14, 16).

The significance of the relationship of streptococcus MG to primary atypical pneumonia is not at all clear, and will probably remain uncertain until the causal agent or agents of this disease are definitely established. Thomas *et al.* (13) have suggested the following possibilities: 1) This microorganism may be merely a secondary bacterial invader; 2) it may possess a purely coincidental antigenic relationship to the causative agent or agents as, for example, do members of the *B. proteus* group to various rickettsiae; or 3) it may act alone or together with some other infectious agent in the pathogenesis of the disease. At the present time the available evidence is insufficient to indicate what rôle this non-hemolytic streptococcus may play in primary atypical pneumonia.

Peterson, Ham, and Finland (19), as well as Turner (20), noted the occurrence of agglutination of human erythrocytes in the cold

with sera of patients having primary atypical pneumonia. Their findings have been confirmed and the significance of the reaction has been studied by a number of investigators (21—25). Cold hemagglutinins appear in the sera of approximately 50—55 per cent of patients with primary atypical pneumonia and occur in other diseases only rarely. They are first demonstrable in a significant titer usually about the 7—10th day after onset and usually reach highest titer between the 14th and 21st days. The agglutinin titer drops fairly rapidly after reaching its maximum so that it may be significantly lower between the 3rd to 5th weeks. A titer of cold hemagglutinins of 1 : 40 or higher is considered significant in this laboratory. The presence of cold hemagglutinins and their titer are in general directly related to the severity or duration of the illness. Unfortunately, in patients who have only a mild illness, their presence usually cannot be demonstrated (15, 21).

Present evidence suggests that cold hemagglutination is probably a non-specific serological reaction; no satisfactory explanation for it has been proposed. In some respects it may be similar to the non-specific complement fixation reaction which is obtained with various lung tissue antigens in this disease as demonstrated by Thomas *et al.* (26). It should be pointed out, however, that these two reactions are mediated by different components of serum. The occurrence of hemolytic anemia (21, 27, 28) is the chief complication in primary atypical pneumonia associated with the unusual presence of extremely high titers of cold hemagglutinins.

Summary.

The laboratory procedures which were employed in the study of sera from 8 patients, each of whom had an unusual form of pneumonia of undetermined etiology, are described. The results obtained indicate that 4 patients possessed cold hemagglutinins in significant titer, while 3 patients possessed antibodies against streptococcus MG in significant titer. With the single patient from whom acute phase and convalescent sera were obtained, a significant increase in titer of both cold hemagglutinins and streptococcus MG agglutinins was demonstrated. These results indicate that this small family epidemic may be identified as primary atypical pneumonia.

Bibliography.

1. Hogeman, Olle: Family epidemic of primary atypical pneumonia. *Acta med. scand.* 1948, *131*: 466. — 2. Finland, M., Peterson, O. L., Allen, H. E., Samper, B. A., and Barnes, M. W.: Cold agglutinins. I. Occurrence of cold isohemagglutinins in various conditions. *J. Clin. Invest.* 1945, *24*, 451—457. — 3. Commission on Acute Respiratory Diseases, Army of the United States: Cold agglutinins in primary atypical pneumonia and other respiratory infections. *Am. J. Med. Sc.* 1944, *208*, 742—750. — 4. Thomas, L., Mirick, G. S., Curnen, E. C., Ziegler, J. E., Jr., and Horsfall, F. L., Jr.: Serological reactions with an indifferent streptococcus in primary atypical pneumonia. *Science*. 1943, *98*, 566—568. — 5. Hirst, G. K.: The quantitative determination of influenza virus and antibodies by means of red cell agglutination. *J. Exp. Med.* 1942, *75*, 49—64. — 6. Hirst, G. K.: The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus. *Science*. 1941, *94*, 22—23. — 7. Mayer, M. M., Osler, A. G., Bier, O. G., and Heidelberger, M.: The activating effect of magnesium and other cations on the hemolytic function of complement. *J. Exp. Med.* 1946, *84*, 535—548. — 8. Pickels, E. G.: An improved type of electrically driven high speed laboratory centrifuge. *Rev. Scient. Instr.* 1942, *13*, 93—100. — 9. Smadel, J. E., Baird, R. D., and Wall, M. J.: A soluble antigen of lymphocytic choriomeningitis. I. Separation of soluble antigen from virus. *J. Exp. Med.* 1939, *70*, 53—66. — 10. Robbins, F. C., Rustigian, R., Snyder, M. J., and Smadel, J. E.: Q fever in the Mediterranean area: report of its occurrence in Allied troops. III. The etiological agent. *Am. J. Hyg.* 1946, *44*, 51—63. — 11. Hirst, G. K., Rickard, E. R., Whitman, L., and Horsfall, F. L., Jr.: Antibody response of human beings following vaccination with influenza viruses. *J. Exp. Med.* 1942, *75*, 495—511. — 12. Rickard, E. R., Horsfall, F. L., Jr., Hirst, G. K., and Lennette, E. H.: The correlation between neutralizing antibodies in serum against influenza viruses and susceptibility to influenza in man. *Pub. Health Rep.* 1941, *56*, 1819—1834. — 13. Thomas, L., Mirick, G. S., Curnen, E. C., Ziegler, J. E., Jr., and Horsfall, F. L., Jr.: Studies on Primary Atypical Pneumonia. II. Observations concerning the relationship of a non-hemolytic streptococcus to the disease. *J. Clin. Invest.* 1945, *24*, 227—240. — 14. Finland, M., Samper, B. A., and Barnes, M. W.: Cold agglutinins. VI. Agglutinins for an indifferent streptococcus in primary atypical pneumonia and in other conditions and their relation to cold isohemagglutinins. *J. Clin. Invest.* 1945, *24*, 497—502. — 15. Horsfall, F. L., Jr.: Primary atypical pneumonia. *N. Y. State J. Med.* 1946, *46*, 1810—1814. — 16. Eaton, M. D.: Serological differentiation of primary atypical pneumonia from virus pneumonia of the psittacosis group. *Proc. Soc. Exp. Biol. and Med.* 1945, *60*, 231—235. — 17. Meiklejohn, G., and Hanford, V. L.: Agglutination tests with streptococcus No. 344 in primary atypical pneumonia. *Proc. Soc. Exp. Biol. and Med.* 1944, *57*, 356—358. — 18.

- Mirick, G. S., Thomas, L., Curnen, E. C., and Horsfall, F. L., Jr.: Studies on a non-hemolytic streptococcus isolated from the respiratory tract of human beings. II. Immunological characteristics of streptococcus MG. *J. Exp. Med.* 1944, *80*, 407—430. — 19. Peterson, O. L., Ham, T. H., and Finland, M.: Cold agglutinins (autohemagglutinins) in primary atypical pneumonias. *Science*. 1943, *97*, 167—168. — 20. Turner, J. C.: Development of cold agglutinins in atypical pneumonia. *Nature*. 1943, *151*, 419—420. — 21. Finland, M., Peterson, O. L., Allen, H. E., Samper, B. A., and Barnes, M. W.: Cold agglutinins. II. Cold isohemagglutinins in primary atypical pneumonia of unknown etiology with a note on the occurrence of hemolytic anemia in these cases. *J. Clin. Invest.* 1945, *24*, 458—473. — 22. Curnen, E. C., Mirick, G. S., Ziegler, J. E., Jr., Thomas, L., and Horsfall, F. L., Jr.: Studies on primary atypical pneumonia. I. Clinical features and results of laboratory investigations. *J. Clin. Invest.* 1945, *24*, 209—226. — 23. Horstmann, D. M., and Tatlock, H.: Cold agglutinins: A diagnostic aid in certain types of primary atypical pneumonia. *J. A. M. A.* 1943, *122*, 369—370. — 24. Turner, J. C., Nisnewitz, S., Jackson, E. B., and Berney, R.: Relation of cold agglutinins to atypical pneumonia. *Lancet*. 1943, *1*, 765—769. — 25. Meiklejohn, G., Eaton, M. D., and van Herick, M.: A clinical report on cases of primary atypical pneumonia caused by a new virus. *J. Clin. Invest.* 1945, *24*, 241—250. — 26. Thomas, L., Curnen, E. C., Mirick, G. S., Ziegler, J. E., Jr., and Horsfall, F. L., Jr.: Complement fixation with dissimilar antigens in primary atypical pneumonia. *Proc. Soc. Exp. Biol. and Med.* 1943, *52*, 121—125. — 27. Ginsberg, H. S.: Acute hemolytic anemia in primary atypical pneumonia associated with high titer of cold agglutinins. Report of a case. *New Eng. J. Med.* 1946, *234*, 826—829. — 28. Stats, D., and Wasserman, L. R.: Cold hemagglutination — an interpretive review. *Medicine*. 1943, *22*, 363—424.

From the Medical Clinic (Chief: Prof. E. Greppi, M. D.) and the
Institute of Hygiene and Bacteriology (Chief: Prof. G. Mazzetti, M. D.)
University of Florence (Italy).¹

Tuberculin Test and Serum Antibodies in the Experimental Tuberculosis and their Behaviour in Order to the Anatomical Evolution of the Disease.

**(Allergometric, Serological and Anatomico-histological
Researches.)**

By

ORAZIO CARERE-COMES and ALBERTO TESI.²

(Submitted for publication November 17, 1947.)

Although allergic and immunitary reactions for tuberculosis have been the object of several studies, they have not yet been entirely considered in all their possible clinical applications, as we have not reached definite and clear conclusions on their diagnostic and prognostic significance on the function of tubercular antibodies, on the relationship between allergic and immunitary manifestations in the tubercular infection.

The researches on clinical cases have cleared many uncertainties and have somewhat dissipated the skepticism of the most part of investigators and physicians regarding the significance of allergic and immunitary reactions in tuberculosis. Notwithstanding the help of the most perfect diagnostic methods the accurate clinical investigation cannot go beyond a certain limit, it cannot offer to the direct observation the dynamism of the cellular reactions; it leaves some uncertainties in the interpretation of the immunitary mechanism, which can be filled only by presumptive hypothetical postulations.

¹ This work has been carried out with the technical and financial support of the Centre for experimental Researches on Prophylaxis and Therapeutics of the infectious diseases, by the University of Florence.

² Viale Cadorna 13, Florence, Italy.

reactivity after its first appearance generally increases in the following days reaching its maximum about 4 weeks after, and then decreasing more or less rapidly till the death of the animals (according to the virulence of the infecting strain).

The absolute intensity of the intradermal test (or rather the threshold of sensibility) is lower in animals sensitized with non virulent bacilli.

As for the morphology of the tuberculin test already the first researches of Koch showed that while in normal guinea-pigs subcutaneously injected bacilli give rise after several days to an abscess usually degenerating into a persistent ulcer with regional adenitis causing the death of the animal, in tuberculinized guinea-pigs a few hours after the inoculation inflammatory oedema appears causing a rapid formation of a scar and subsequent recovery after a few days, even before the lymph gland could react. (Koch's phenomenon.)

Several investigators have studied the duration of the preallergic period in animals infected with Koch bacilli of different strains and virulence. Thus when in the histopathological picture of human and experimental tuberculosis were recognized different well defined evolutive stages, it was tried to identify these stages as an equivalent to the parallel development of the allergic immunitary state of tubercular infected organism (*i. e.*: the classifications in correlation with the three Rank's stages).

Later numerous researches were carried out in several medical and biological Institutes (Rockefeller Institute for Medical Researches, Forlanini Institute, Davos Sanatorium) with the aim to investigate

1) Whether, although toxicity and virulence are certainly a property only of alive bacilli, the sensibilizing (*i. e.* the capacity to produce the allergic reactivity in the tissues) can subsist without any reference to bacillary virulence.

2) Whether it is possible to identificare in the bacillary bodies or in their culture medium any chemical fraction particularly able to stimulate; the production of different types of tuberculous granuloma (Sabin, Doan and co-workers, Omodei-Zorini, Daddi, Roulet and co-workers).

It was then possible to extract from the bacillary bodies, a phosphatide which was able to reproduce the specific granuloma in animals (Roulet); its stimulating power would be more precisely referred to fatty acids (Tuberculo-stearic and ptyoic

acids) which take part in the molecular structure of this phosphatide.

According to Doan, Sabin and Forkner, in the tubercular granuloma the phosphatide partigens are probably accumulated in the giant and epithelioid cells; *i. e.* in the cellular elements where the bacilli bodies are mostly found.

The experimental inoculation of the phosphatide partigen was not able to sensitize the skins of the animals to the tubercular antigens, but produced a miliary tubercle dissemination in the visceral and lymphatic organs; the tubercles were mostly formed of giant and epithelioid cells; and the blood picture showed in the same time a marked increase of the lymphocytes/monocytes ratio.

This ratio (already recognized in the preagnostic significance and its inmost essence by earlier clinical observation) appeared thus undoubtedly as the logical equivalent of outspreading of tubercular toxins and therefore a sign of unfavourable prognosis.

Also in the interesting researches carried out by Birkhaug on the tubercular allergy and immunity it is experimentally pointed out that the rising of the monocytes in the blood picture is the first peripheral phenomenon of virulence, to which suddenly follows a decrease of lymphocytes. The lymphocytes/monocytes ratio decreases proportionally to the severity of the infection.

Therefore in experimental tuberculosis Birkhaug observed the least marked decrease of the above mentioned ratio in the previously vaccinated or later vaccinated and desensitized (yatergic) guinea-pigs.

As a matter of fact the animals treated with phosphatide partigens the organs of which were widely invaded with specific epithelioid granuloma submitted to infection with virulent bacilli were much less resistant than untreated control animals.

A rise of specific resistance instead was noticed in animals after treatment with killed or attenuated germs; it was in this manner possible to produce in the animals specific skin sensitisation visceral granulomata of a mostly lymphatic or fibrous type, decrease of the monocytes/lymphocytes ratio in the peripheral blood.

A less marked significance in the pathogenesis of the tubercular disease was shown by the other Chemical fractions obtained from the bacillary bodies or from their culture media. The waxes would only act as an irritating foreign body non-specific stimulus. The purified protein fractions, extracted from culture media (Sabin and Coll) showed marked tuberculinic activity in healthy guinea-

pigs; they acted not in a toxic but in a sensitizing way. The sensibilization in any way was an anaphylactic one. The purified proteins were namely not able to produce either allergic reactivity of the skin or immunizing antibodies against the tubercular toxins (Birkhaug).

The polysaccharide fraction would be scarcely toxic and only in animals with diffuse tuberculosis; their antigenic power, so far not recognized, has been lately confirmed by Daddi.

Summarizing, the researches so far accomplished showed that:

1) The histopathological results of experimental tuberculosis may be considered as the right morphological equivalent of those immunitary processes of which the specific allergic reactivity is based; in fact their occurrence, intensity and development are closely connected with the concurrent serum immunological reactions showing the immunitary defence of the infected animals.

2) Together with the immunitary factors another fundamental element determining the development of the specific granuloma is the bacterial charge (number and virulence of infecting bacteria).

3) In case of weak allergic reactivity and elevated bacterial charge the specific toxins (and particularly the phosphatide fraction) impregnate the connective tissue cells (which assume the typical epithelioid or giant cell aspect), the skin allergy is deficient or absent and the disease shows a fast and spreading evolution.

In case of strong allergic reactivity and deficient bacterial charge, the bacillary toxins are able to infiltrate the connective tissue-cells (they are namely scarcely produced from the germ) or are nearly completely neutralized by the prevalent defensive capacities of the infected tissues; the connective tissue reaction appears, as in all chronic infections, as a lymphoid and sclerotic one; the skin allergy is marked; the bacterial foci gradually become isolated and circumscribed by the connective tissue reaction; the whole process tends therefore to improvement.

The last mentioned conclusions summarize the main points of our knowledge; *i. e.* the relationship between the pathological picture and the immunitary allergic phenomena, as resulting from researches on experimental tuberculosis; the limits amongst the referred possibilities are not practically so clear cut owing to the presence of all those intermediate stages which can result from the variable rate of immunitary reserves on one hand and bacterial charge on the other.

Data have also been accumulated concerning the relationship between the presence of circulating antibodies and the histopathological picture of experimental tuberculosis; anyway systemic researches on the behaviour of complement fixing antibodies in the different stages of experimental tuberculosis are lacking.

The actual references on the specific and diagnostic meaning of the C. F. R. are rather contradictory which can be related to a missing evaluation and standardization of the C. F. R. for tuberculosis that is usually differently executed and interpreted by the various investigators. For instance while Richter and Lurse using Wassermann antigen obtained 100 % positively in bovine tuberculosis, Wendt obtained positive results also in healthy animals (post-mortem control), Lurse on the other hand obtained negative reactions in infected rabbits, guinea-pigs and birds.

Hruska and Pfenniger found C. F. R. positive in 85 % of infected oxen especially in the widely disseminated forms; Brocq and Rousseau and co-workers found 95 % positivity in infected oxen, particularly intensive reactions in purulent and caseous forms.

Also in healthy oxen are to be found with a certain frequency (2.2 %) C. F. antibodies in the blood according to Hruska and Pfenniger more often (11.1 %) according to Panisset and Verge. Considerably more specific results were obtained instead in tuberculous dogs (Verge and Urbain). The experimental researches of Nagel on different groups of vaccinated and successively superinfected animals came to unsatisfactory conclusions as far as the diagnostic value of the C. F. R. is concerned. The C. F. R. executed (with Witebsky's and Klingenstein's antigen) were in fact, according to Nagel, scarcely specific and variously correlated with anatomical picture of the disease. More effective results were obtained by Satta and Buonomini independently by the bacterial charge, positive C. F. R. appeared only consequently to the development of the first inflammatory reactions of the tissue, at any rate in those animals that showed rather wide spread anatomical involvement and efficient defensive power (stronger positivity amongst the previously vaccinated ones).

Being the tubercular focus a circumscribed one the C. F. R. was usually negative; sometimes it resulted positive also in circumscribed caseous Tbc., however it was preceded by the appearance of the skin allergy (Buonomini). The researches on the behaviour of the C. F. antibodies in experimental tuberculosis, especially

in guinea-pigs, are sometimes frustrated by the interference of anticomplement power in the blood serum (Nagel).

Conclusively, our knowledge on the significance of the C. F. R. for tuberculosis are still rather incomplete and often disagreeing each other; the anatomical basis, which the appearance of C. F. R. can be referred to is practically unknown.

As regarding the prognostic significance of the circulating antibodies, it is generally supposed that their occurrence indicates the evolutive tendency of the diseases. Amongst the diagnostic tests for tuberculosis (skin allergy, C. F. R., opsonic index, enzyme reaction etc.), only the first is able to give a faithful estimation of the specific resistance against tuberculosis as resulted from the congruous investigations conducted by Mazzetti and co-workers, being the antitubercular resistance calculated studying the survival rate of the animal and on their capacity to destroy more or less rapidly the circulating injected bacilli.

Experimental Materials and Methods.

In order to arrive at definite conclusions about the significance of both tissue and blood antibodies and their reciprocal interrelations, in the singular stages of experimental tuberculosis, we searched concomitantly in our experimental animals the concurrent modifications of weight, of skin allergy, of the circulating antibodies and through periodical investigations, the correlated different aspect of the concomitant anatomical evolution of the disease.

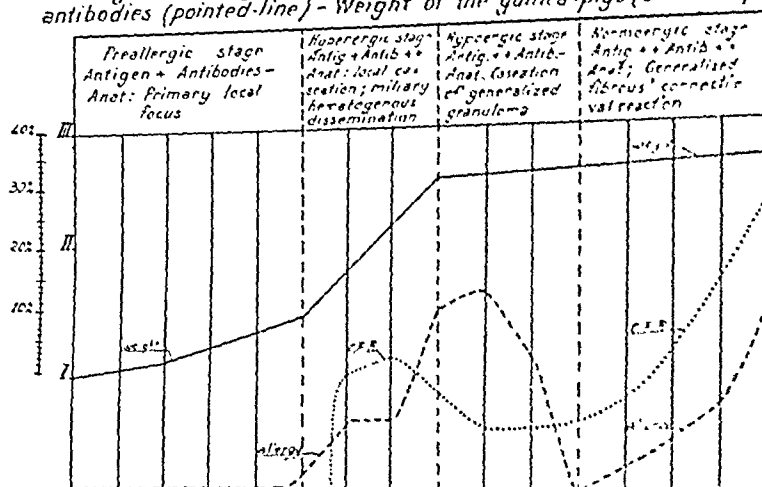
We paid attention that the infecting bacillary dose was small enough to prolong as much as possible the course of the disease.

We selected guinea-pigs coming from the same breed, of the same age and weight and we grouped them in five lots each of 10 animals of the same sex.

All animals received the established bacterial charge consisting of highly diluted sputum (1/1000—1/10000), previously recognized as containing a large amount of bacilli of human tuberculosis.

The groups of guinea-pigs were injected subcutaneously in the left thigh with 1 ml of 1/10000 dilution of bacterial suspension; two groups were injected intratracheally with 0.1 ml of 1/1000 diluted bacterial suspension. In this way each animal, subcutaneously or intratracheally infected, received the same amount

Table I - Subcutaneous infection
 Allergic reactivity of the skin (treated-line) and rate of serum antibodies (pointed-line) - Weight of the guinea-pigs (uninterrupted-line)



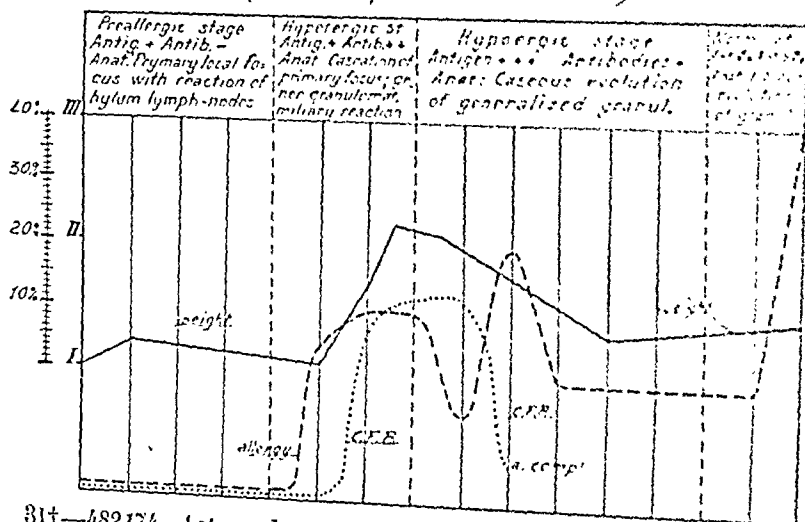
On the abscisses the time in weeks from infection -

On the ordinates allergometric values (I = pap 10 mm.; II = 20 mm.; III = 30 mm. etc.) and rate of serum antibodies (I = C.F.R. pos. dil. $\frac{1}{10}$; II = pos. dil. $\frac{1}{10}$; III = pos. dil. $\frac{1}{10}$ etc.) On the left: scale in % of average weight of the animals

of tubercle bacilli, i. e. a constant infecting charge of about 56 bacilli as resulted from the count of Petragnani culture medium inseminated with the same amount of bacterial suspension that was used for the experimental infection of one animal.

We followed the course of the disease in the succession of different stages observing aspect, weight and also the anatomical

Table II - Intratracheal infection
 (For interpretation see Table I)



evolving of the disease as resulting from weekly post-mortem control of the singular animal of one group.

Concomitantly we executed weekly an intradermal tuberculin test in each animal and we evaluated the local reaction 48 hours after the injection, observing also the rate of the reaction on the basis of the extent of the external reaction or of the highest tuberculin dilution able to induce a reaction; as antigen we used purified proteins (M. D. C., prepared in the Forlanini Institute).

The C. F. R. was also practised weekly in the blood. Some trials to determine the presence of C. F. antibodies in organ extracts were unsuccessful owing to their strong anticomplement power.

Results.

Notwithstanding the very scarce infecting material charge not even a single animal is spared from contracting tuberculosis.

Therefore the possibility to produce a slowly progressive and uniform evolution of the disease was satisfactorily obtained according to our experimental design.

A particular relation on the evolvement of the experimental infection, allergy, C. F. R. and on the anatomical and histological results, as they were observed by the singular animals, would assume a too large extent. Otherwise owing to a satisfactory conformity of the experimental pictures obtained in the different animals, the clearness of the exposition will be not particularly damaged being only generalized and summarized in the short limits of the following reports.

Subcutaneous Inoculation.

Five weeks after the infection, it was remarked the appearance of a primary focus just in the left thigh muscles, *i. e.* at the injection point, with associated granulomatous sometimes caseous involvement of the local lymph nodes.

The latter became more evidently caseous in the following days, during which a miliary hematogenous dissemination in the spleen and lungs took place. At the eighth week also the hematogenous foci in the spleen assumed larger extent and caseous transformation, while the connective tissue of the spleen is generally stimulated and induced to the fibrous proliferation and following splenomegaly.

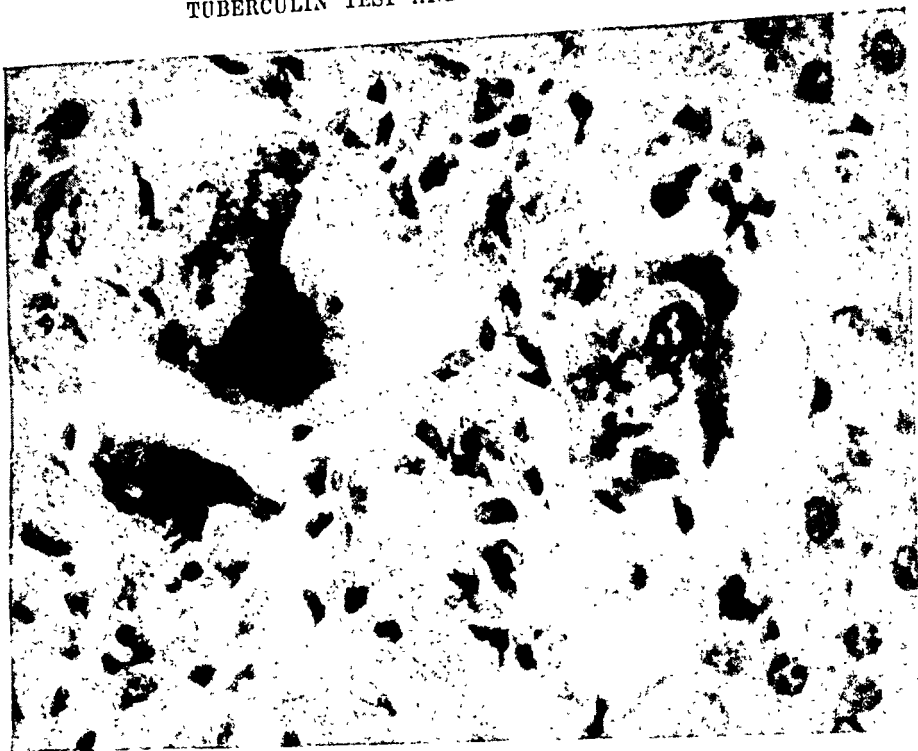


Fig. 1. (625 X)

In the liver the miliary tubercles are also proliferating, but appear not yet affected by caseation; their aspect indeed is also in this stage a prominently cellular one, wherever lymphatic and giant cells are the most participating ones (Fig. 1).

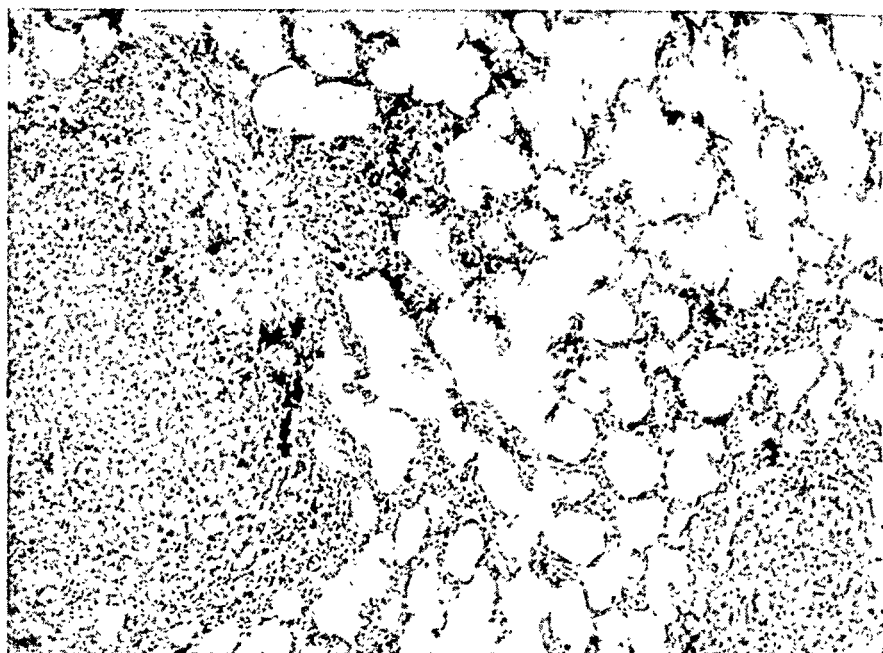
The tubercular granulomatous or caseous infections involve also the lymph nodes of the lumbar retroperitoneal groups.

Successively the diffusion of the tubercular process and its tendency to the caseation go on, owing to an ascending propagation in the prevertebral lymph nodes, to the tracheo-bronchial and cervical lymph nodes (about after 12 weeks).

Also the splenomegaly extent is increasing while its specific foci become also larger and more caseous.

Intratracheal Inoculation.

The intratracheally inoculated guinea-pigs showed sometimes already at the second week the appearance in the lungs of a primary focus; *i. e.* a circumscribed exsudative alveolitis surrounded by miliary tubercles (Fig. 2) and associated with a granulomatous reaction of the hilum lymph nodes. After 4 weeks the pulmonary

Fig. 2. (115 \times)Fig. 3. (200 \times)

in some places other forms. The number of well preserved *lymphocytes* and *lymphoblasts* is much smaller than in the normal lymphadenogram. This is explained by the increased number of Gumprecht's shadows. *Reticulo-endothelial* cells were not observed, neither were *granulocytes* seen; some erythrocytes are present.

Chronic myeloid leucemia. The *erythrocytes* when compared with a normal lymphadenogram show no differences. The *granuloblastic system*: In all fields of vision there are several or so typical myeloblasts (Fig. 12), and somewhat smaller proportion of metamyeloblasts and promyelocytes, and still smaller number of neutrophil myelocytes, metamyelocytes, stab cells and polymorphs. Only adult forms of oxyphil and basophil granulocytes were observed. The majority of the cells were well preserved, the rest however showed the cytoplasm to be more or less damaged with partial and even complete separation from the nucleus. From these separated parts the basophil fragments of cytoplasm arise. They are usually spherical, of different size, from that of normocyte to small round granules, which at times fill the entire field of vision. The nuclei deprived of cytoplasm undergo disintegration giving origin to *Gumprecht's shadows*. *Lymphocytes* are less numerous than in a normal gland and typical *lymphoblasts* only exceptionally were observed. Typical *reticular cells* appear as a rule in the same proportion as in normal lymphadenogram. Many cells in apparent transition from reticular cells to myeloblasts were observed. *Plasma cells* were scarce.

Acute myeloid leucemia. On examining the smears from enlarged cervical glands the following was confirmed: In the *erythroblastic system* scanty nucleated red cells and a few erythrocytes with fairly marked aniso- and poikilocytosis were seen. *Lymphocytes* and *lymphoblasts* were far less numerous than in normal lymphadenogram. In the *granuloblastic system* there were many typical myeloblasts (Fig. 13), often showing signs of degeneration and disintegration with development of Gumprecht's shadows. There were no more differentiated forms — from metamyeloblasts to adult granulocytes. A few cells with the characteristics of *reticular cells* were seen; their structure is on the whole similar to that of myeloblasts, but they are a little larger.

Our material, though it is very modest, allows us to draw the following conclusions concerning the value of the lymphadenogram in diagnosis of leucemia: The lymphadenogram in the course of chronic lymphatic leucemia, in leucemic as well as in aleucemic

until the 8th and 9th week when the animals showed the strongest reactivity.

At this point started the 3rd stage of the experimental disease, a stage sharply delineated from the complete disappearance of the skin allergy at the 10th week and from the other immunitary and pathological modifications, which we will later mention. The disappearance of the specific allergy was, however, only a temporary one: already after a period of two weeks, the animals became again allergic and more and more intensively, reaching the highest allergic level that was ever attained by them.

The concomitant correlated investigation of the anatomical picture demonstrated that allergic reactivity came upon the stage in a time when only the primary lymphatic focus had revealed itself, and more precisely immediately following the appearance of the anatomical focus.

Afterwards the reaching of the highest allergic level the end of the second stage appears as corresponding to the most extensive involvement of the lymph nodes. The successive predominating caseation of most tuberculous foci likely was the cause of the fall of the allergy in the 9th—11th week; whilst its new arising can be referred to the sclerotic evolution of the connective tissue proliferation and perhaps also to a backward outcoming granulomatous reaction in the not yet affected, tracheal and cervical lymph nodes. The mentioned coincidences between our allergic respectively anatomical results were not considered presumptively as obligatory casually connected and was not left out the supposition that they could be quite fortuitous. As we will see further on the experience gained in the further observations, strengthened more and more the meaning that all allergical immunitary and pathological manifestations were intimately connected one another, quite constantly, assuming in effect the significance of different expressions of a unitary fundamental defensive mechanism.

The participation of the whole organism to this process appears both from the evident parallelism between allergic reactivity and body weight of the animals and the different stages of the disease (see table I). Particularly significative is the coincidence of the strongest weight increase with the appearance and suddenly arising of the allergy in the second hyperergic stage of the infection. In this period, *i. e.* from the 6th to the 9th week the general pathological picture is dominated from the defensive mechanism

b) Intratracheal Infection.

1. *Preallergic stage*: scarce antigen; missing antibodies, primary exsudative pulmonary infection with granulomatous hilum-lymphadenitis (1—4th week).

2. *Hyperergic stage*: scarce antigen, exuberating antibodies, miliary tubercle dissemination, caseation of the hilum lymph nodes; strong allergy, intense but delayed C. F. R., increasing weight (4—7th week).

3. *Hypoergic stage*: exuberating antigen, scarce antibodies generalized caseous, evolutive tuberculosis, decreasing allergy, tubercle dissemination (anticomplementary of serum), decreasing of weight (7—13th week).

4. *Normoergic stage*: exuberating antigen and abundant antibodies, strong allergy, anticomplementary of serum, increasing weight (after the 13th week).

References.

Birkhaug, K.: Allergy and immunity (iathergy) in experimental tuberculosis. X. Variations in the blood cells of allergic, iathergic (desensitized) and control guinea-pigs during a virulent tuberculous infection. Acta med. scand. CX, 201—229, 1942. — Birkhaug, K.: Concurrent development and subsequent dissociation of anaphylaxis, allergy and immunity in tuberculosis. Acta med. scand. CXII, 393—425, 1942. — Boquet, A. and Nègre, L.: in «Calmette, A.: L'infection bacillaire et la tuberculose. Masson. Paris 1936.» — Brocq-Rousseau, Urbain A. and Cauchemez: La réaction de déviation du complément appliqué au diagnostic de la tuberculose bovine. Ann. Inst. Pasteur, 37, 872—878, 1923. — Buonomini, G.: Ulteriore contributo alla conoscenza del valore antigene del Fenbattacin alta (Petragnani) per la reazione di fissazione del complemento nella tubercolosi. Sperimentale, 94, 702—710, 1940. — Daddi, G.: Il bacillo di Koch. Cappelli ed. Bologna. 1938. — Hruska, Ch. and Pfenniger, W.: Le diagnostic de la tuberculose chez les bovidés au moyen de l'antigène de Besredka. Ann. Inst. Pasteur, 35, 9, 101, 1921. — Luhrs: cited by Sivori, op. cit. pag. 268. — Mazzetti, G. and Vigni A.: Sul significato della persistenza in circolo del bacillo di Koch negli animali inoculati per via endovenosa. III. Sperimentale, 94, f. 9, 545—557, 1940. — Nagel: cited by Schulte-Tigges, op. cit., pag. 502. — Omodei-Zorini, A. and Daddi G.: Lotta contro la tbc., 5, 1181, 1934; 6, 27, 1935, cited by Daddi. — Panisset, L. and Verge, J.: La réaction de fixation dans l'entérite hypertrophiante des bovidés au moyen des antigènes tu-

Banting and Best Department of Medical Research University
of Toronto (Canada).

The Lipotropic Factors.¹

By

C. H. BEST, F.R.S.

(Submitted for publication December 2, 1947.)

It was not my good fortune to know Professor H. C. Jacobæus personally, but many of my friends in Norway, Sweden, and Denmark have told me of his distinguished and stimulating career. In 1943 Professor Key discussed the contributions of Professor Jacobæus to medical research. I have been greatly honoured by the invitation to give the second lecture under the auspices of the H. C. Jacobæus Foundation.

The studies of investigators interested in the physiology and biochemistry of fats have been facilitated by the discovery, during the past quarter of a century, of a number of substances which exert »lipokinetic» effects, i. e. their administration profoundly affects the rate of transport of fat from liver to depôt or in the reverse direction. Our group in Toronto has been particularly concerned with three of these: insulin, choline, and the liver fat increasing substance in certain anterior pituitary extracts. For this reason, and because of our continued interest in the many unsolved problems of fat metabolism, I have selected »The Lipotropic Factors» as the title of my Jacobæus Lecture.

If I may digress for a moment to discuss terminology, it is obvious that what I am calling physiological lipokinetic agents, may be of either hormonal or dietary nature. The hormone, insulin, has profound effects upon fat mobilization. When an animal is suddenly deprived of its pancreas the fat depôts rapidly melt

¹ The Nordisk Insulinfond Jacobæus Lecture for 1947, delivered at the University of Copenhagen on September 4th.

away and the liver becomes large and very fatty. This excess deposition of fat in the liver which is observed even in fasted animals is almost certainly due in part to transport of fat from the depôts. When insulin is administered there is prompt alleviation of the ketosis, elimination of excess fat from the liver and a lowering of blood fat. The depleted fat depôts are rapidly restored. These findings were reported from Toronto soon after the antidiabetic hormone became available (1). The work of Bouckaert (2), of Drury (3), and the more recent findings of Stetten and Klein (4), provide evidence that insulin accelerates the formation of fat from carbohydrate, *i. e.*, it possesses *lipogenic* as well as *lipokinetic* properties. These two effects may, of course, be very intimately associated.

The liver fat increasing principle of the anterior pituitary exerts an effect on fat mobilization which is opposite to that of insulin, *i. e.*, in the fasting animal it increases the rate of transport of depôt fats to the liver (5, 6). This »adipokinetic action», as Weil and Stetten (7) have termed the effect of the pituitary factor is, in part, prevented by the administration of insulin and is greatly reduced by the provision of food (8).

We are concerned primarily to-day, however, with the third group of lipokinetic factors — choline and its precursors. In 1935 we suggested calling these substances »the lipotropic agents» and defined the term with reference to the ability of such compounds to prevent the accumulation of excess fat in the liver or to accelerate its removal. Since it has been established that the dietary factors which prevent the kidney lesions in young rats also prevent deposition of fat and cirrhotic changes in the liver, and since, in general, the factors which increase deposition of fat in the liver aggravate these other changes, *i. e.*, cirrhosis and the kidney damage, we now suggest that the term »lipotropic» should be broadened to include the prevention or cure of all the abnormal changes which are produced in the body by a specific dietary deficiency of choline or its precursors. Only those effects of methionine which are exerted through formation of choline would be included under this definition.

The large fatty livers observed in depancreatized dogs which are maintained for long periods on adequate amounts of insulin, may be prevented by the addition of raw pancreas to the diet or, as was shown by Hershey and Soskin, in Toronto, by crude lecithin. In an extension of this work, Huntsman and one of us (C. H. B.)

showed that purified lecithin prevented fat deposition in the livers of rats fed diets rich in fat. They subsequently identified choline as the active component of lecithin (9). In the same year they showed that betaine, another naturally-occurring quaternary ammonium base, exerted a similar but weaker action.

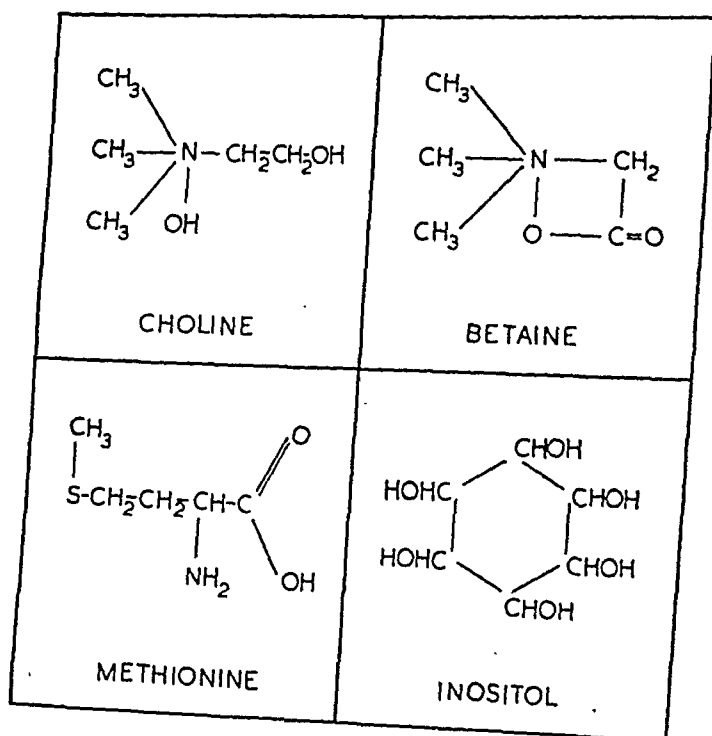


Fig. I.

Several years later an improved bio-assay procedure made possible the recognition that many articles commonly used in basal diets contained an appreciable amount of choline. Thereafter, it was possible to eliminate undesired choline from the basal rations and to demonstrate much more clearly the effect of added choline. The lipotropic effect of casein was first noted in Toronto (10) and the results were discussed with Professor Channon in Liverpool, who, with his colleagues, greatly extended the work to include studies of many other proteins (11). It remained, however, for Tucker and Eckstein to discover, in 1937, the lipotropic activity of methionine (12). In 1941, Gavin and McHenry (13) reported that inositol exerted a lipotropic effect in fat-free diets. Thus, four naturally-occurring substances have been identified as dietary components which effect the deposition of the fat in the liver (Fig. I).

When du Vigneaud and his colleagues (14) discovered trans-methylation they supplied an explanation for the similar lipotropic activity of compounds as unlike as methionine and choline. They showed that the labelled methyl group of dietary methionine could be found in the choline of liver lipids. The question naturally arose as to whether the labile methyl group or the choline molecule was the effective lipotropic agent. In 1942, Welch and Landau established that arsenocholine is lipotropic but that it does not possess labile methyl groups. They presented a strong argument for the view that the intact choline molecule is the effective lipotropic agent when methionine, betaine or choline is fed. Stetten (16) had already shown, by feeding choline labelled with heavy nitrogen, that dietary choline is rapidly incorporated into the molecule of liver lecithin and that ethanolamine serves as the methyl acceptor from which choline is formed when compounds such as methionine or betaine are fed.

McArthur in our laboratory has recently shown that the compound colloquially known as »triethyl choline» when ingested, is incorporated into the molecule of liver phospholipids. This compound possesses no labile methyl groups but it exerts a moderately strong lipotropic effect. This work supports the contention of Welch that the intact choline molecule rather than the labile methyl group is the effective lipotropic substance.

There is considerable evidence that the mechanism of action of choline and its precursors is to accelerate the rate of turnover of the liver phospholipids. The acceleration, by choline, of the rate of incorporation of inorganic phosphorus in the liver phospholipids, was first shown by Perlman and Chaikoff (18) using radioactive phosphorus as a tracer. Boxer and Stetten (19) have shown that ingestion of choline accelerated the turnover of choline in liver lecithin. Thus it appears that the turnover rates of two constituents of the molecule of liver lecithin are accelerated by dietary choline. It has been assumed that the same effect is exerted on the fatty acids of the liver lecithin and that this explains the increased rate of transport of fat from the liver when choline or its precursors are administered, but no direct proof has as yet been supplied by actual measurement of turnover rate of the liver fatty acids. This data and the results of further quantitative measurements *under equilibrium conditions* of the turnover rates of the other components of liver phospholipids are urgently needed.

The mechanism of action of inositol remains a mystery. Folch and Woolley (20) found inositol in phospholipid isolated from the cephalin fraction of brain. MacPherson, in our laboratory, has shown that inositol is present also in the cephalin fraction of phospholipids from rat livers. The fact that inositol does not protect the kidneys of young rats fed a hypo-lipotropic diet (21) (22), and the recent finding of Lucas, Ridout and Patterson in my laboratory that inositol exerts a negligible effect on liver fat when normal amounts of fat are present in the diet, suggest that this substance has little significance as a dietary lipotropic agent under physiological conditions.

The importance of the nature and the amount of protein in the basal diet used in lipotropic studies, has been stressed by the results obtained in Channon's laboratory, in Eckstein's and in our own. Beveridge, Lucas and O'Grady (23) found that the adequacy or otherwise of the diet with respect to essential amino acids may alter, or even reverse the findings which are obtained in certain types of lipotropic studies. Griffith and Mulford (24) had earlier shown the importance of the total amino acid sulphur, in the diets of young rats. Our observations are fully in accord with their statement that dietary factors which improve food consumption and rate of growth, may cause fatty livers or hemorrhagic kidneys in young animals, without themselves being possessed of any essential anti-lipotropic activity. The addition of cystine to a basal diet improves the supply of sulphur and permits more growth. In the absence of labile methyl groups the deficiency of choline is aggravated and still more fatty livers may result. The so-called antilipotropic effect of cystine or of biotin, may be, in large part, explained along these lines. Handler (25) has shown that the addition of a balanced salt mixture to a basal diet devoid of minerals, may produce a marked increase in the rate of growth and simultaneously a development of very fatty livers. The severe mineral deficiency of the basal diet prevented growth and under such conditions fatty livers did not develop even in the absence of choline. (We may add here, parenthetically, that the effect of lack of choline may be readily demonstrated in spite of a considerable loss of weight.) The explanation of Handler's results doubtless lies, as Treadwell has suggested, in the fact that the basal diet which contained 15 % casein, provided enough methionine to supply labile methyl groups for adequate production of choline when none of the methionine was required for growth.

A similar explanation probably accounts for the anti-lipotropic effect of thiamine observed by McHenry, and of thiamine, riboflavin and pantothenic acid observed by Engel.

Methionine is a versatile metabolite; (a) being an essential amino acid, it is required as such, for maintenance and growth, (b) some is used to supply sulphur for formation of cystine, bile acids, etc., and (c) some may be used to supply labile methyl groups for the biosynthesis of choline. The work of several groups has suggested that methionine may be utilized preferentially for maintenance and growth and only after these requirements have been satisfied is any available for lipotropic action. A possible explanation for the failure of methionine to exert any lipotropic effect under the conditions recently described by Rose, Machella and György (26) is that utilization of almost all of the methionine in an attempt to maintain body substance, leaves scarcely any for lipotropic action.

In the recent studies which Prof. C. C. Lucas and I have carried out in collaboration with Dr. Ridout and Dr. Patterson, we have attempted to take advantage of the advances in our knowledge of the lipotropic factors, to conduct a reinvestigation of the relative lipotropic potencies of various natural and synthetic compounds which affect the accumulation of fat in the liver. For these studies the basal diet should be adequate in all respects, save for the lipotropic factors, and these should, if possible, be entirely absent. The essentiality of methionine, however, seems to us to preclude its complete removal from the diet and further more no readily available protein entirely free from methionine is known. Since it was not considered feasible to use purified amino acids in the experiments in which large numbers of rats must be maintained, a series of studies with graded doses of the various lipotropic agents, have been conducted on rats fed diets with a low methionine content. The basal diet is low in methionine but is believed to be adequate with respect to the other essential amino acids.

Choline was seen to exert a marked lipotropic effect in fat-free diets. Under these conditions, approximately 6 milligrams per day was the smallest amount which produced a maximum effect, *i. e.*, the saturation dose. Inositol also exerted a lipotropic effect in fat-free diets, the saturation dose being about 3 milligrams. It was observed, however, that this maximum effect of inositol leaves the liver fat nearly three times as high as that obtained

when the optimum amount of choline is fed. When 12 % or 30 % of fat is substituted for an equal amount of sucrose, the lipotropic effect of choline was almost the same as that obtained on the fat-free diet. Inositol, however, has exerted no demonstrable effect when either 12 % or 30 % of fat was used.

Since our basal diet was low in organic sulphur, cystine was added in some experiments. As anticipated, growth was improved and a greater deposition of liver fat was observed in the control group — 34 % as compared to 24 %. The animals were group pair fed. Choline caused an abrupt fall but slightly more was required to produce a maximum effect. Inositol was without action.

In confirmation of previous findings the addition of cholesterol to basal diets increased the demand for choline. This is due largely to the resistance of the cholesteryl esters to the lipotropic effect. Inositol has no selective effect on cholesteryl esters and, in fact, under many conditions has no action in preventing deposition of either neutral fat or cholesteryl esters in the livers of cholesterol fed animals.

Hemorrhagic Kidneys of Choline Deficiency.

As Griffith and Wade (27) originally showed, a diet sufficiently low in choline and its precursors, methionine and betaine, produces fatal kidney lesions with great consistency in young rats within seven to ten days. The gross changes in liver and kidneys are illustrated in Fig. II. With diets drastically low in the lipotropic factors these lesions may be produced in much older rats. The pathological picture has been described by Christensen (28), by György and Goldblatt (29), and by others. For our discussion on fat metabolism to-day the primary interest in these kidney lesions is their possible relationship to abnormality of fat deposition or utilization. Griffith has noted in his review in «Biological Symposia» (30) that fatty changes were observed by Christensen in the cells of the proximal convoluted tubules of the kidney. A detailed study of this aspect of the kidney pathology has recently been made by Hartroft in our laboratory. He has found very definite signs of fatty degeneration in the cells of the proximal convoluted tubules but it remains to be decided whether or not this alteration in fat metabolism is responsible for any part of the subsequent degenerative changes in kidney tissue. Dessau and Oleson (31) have recently suggested that the primary lesion in

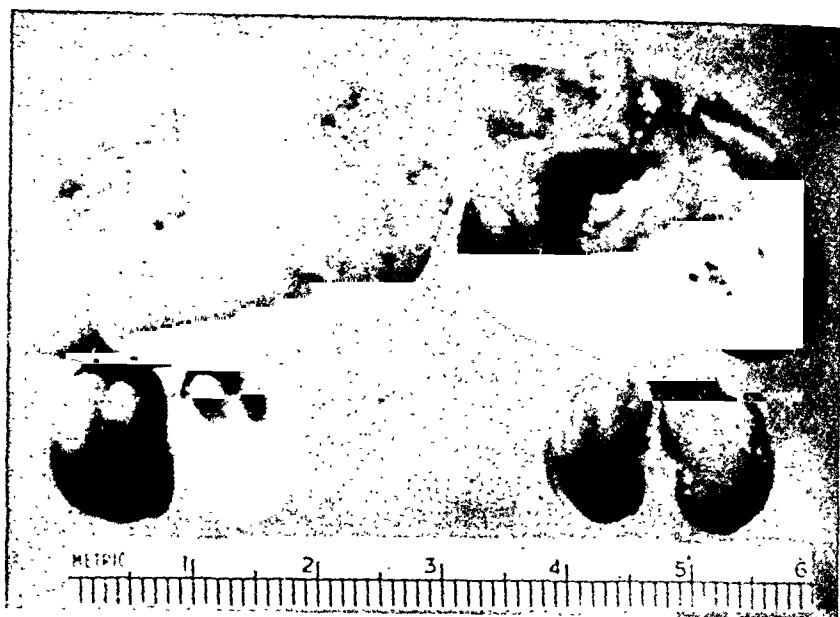


Fig. II. In young rats 7 days of choline deficiency produces these dramatic changes in liver and kidneys.

the kidneys is vascular. It has been shown that there is a fall in phospholipid content of kidney in choline deficiency, and that the rate of turnover of radio-active phosphorus in the phospholipids of this tissue is accelerated by the administration of choline (32).

The Cirrhosis of Choline Deficiency.

It is now well established, as mentioned above, that the fatty liver of choline deficiency in diabetic dogs or normal rats, if allowed to persist, progresses to a definite cirrhosis, which is similar to that not infrequently observed in human subjects. This work has been carried out by Chaikoff, Connor and Biskind (33) in dogs, and by György and Goldblatt (34), Lillie, Daft and Sebrell (35), Blumberg and McCollum (36) and Webster (37) in rats. It is agreed that choline is a specific in the prevention of this type of experimental cirrhosis and its effect on cirrhotic changes which may be produced by the administration of various industrial poisons, has been tested. These latter studies, many of which have been conducted in the laboratories of the U. S. Public Health Service by Neal, Daft and their colleagues, have shown that

choline and methionine, the latter of which provides both labile methyl and sulphur, exert a measure of protective action. Sellers in our laboratory has been studying the rôle of the lipotropic agents in the treatment of carbon tetrachloride cirrhosis in rats. Choline and methionine produce profound curative effects, as indicated by the disappearance of excess fat, regeneration of parenchymal cells, and an apparent decrease in the fibrous tissue. It is necessary to have a diet extremely high in methionine rich protein to duplicate the effects of optimal amounts of choline or methionine. When the lipotropic factors were not made available there was no evidence of recovery. Inositol appeared to decrease the mortality rate of the animals, but exerted no favourable effect on fat deposition or on cirrhosis.

A very high incidence of tumours has been reported by Copeland and Salmon (38) and by Engel, Copeland and Salmon (39) in rats on diets low in the lipotropic agents. Animals receiving choline were free of tumours. This probably indirect effect of choline deficiency will undoubtedly receive much further study.

In the concluding section of this short review I should like to refer again to the original observations on the protective effects of raw pancreas added to the diet of insulin-treated depancreatized dogs. Among the many possibilities which were suggested as an explanation of this effect, two have been most actively investigated. We have described the action of the dietary factor choline, which was present in sufficient amounts in the large supplement of pancreas which was fed to the depancreatized dogs to exert a favourable effect. The second possibility, that of the rôle of the pancreatic enzymes, has been painstakingly investigated by Chaikoff and his group in California. Ralli, Rubin and Present (40), Montgomery, Entenman and Chaikoff (41), and others, have shown that ligation of the pancreatic ducts may lead to fatty changes in the liver. Chaikoff and his collaborators have prepared an active fraction of the pancreatic tissue which is effective in preventing fatty livers in depancreatized dogs, but does not owe its activity to choline or its precursors. More recently they have shown that an effective agent is present in pancreatic juice, and they have suggested that the action of their anti-fatty-liver material is enzymatic in nature. Even more recently Chaikoff, Entenman and Montgomery (42) have shown that hydrolyzed casein or free methionine exerts a marked lipotropic effect in depancreatized dogs, an action which is not produced by unhydro-

lyzed casein. Thus the proof is practically complete that pancreatic enzymes do play an important rôle in the effect of pancreas fed to depancreatized dogs. Methionine is liberated which, by methylation of amino-ethyl-alcohol, increases the amount of choline available. Palmer (43) in our laboratory and Chaikoff and his collaborators have isolated several crystalline proteolytic enzymes from the active fraction of pancreas. If we may assume, as it now appears justifiable to do, that this active fraction is a proteolytic enzyme it remains only to determine which of these are effective in producing the liberation of methionine.

We have thus seen that lipotropic agents produce very definite effects on fat transport and deposition. In the absence of these factors in young animals liver and kidney function is damaged and the metabolism of protein, fat and carbohydrate may be seriously affected. Protein metabolism through methionine is now inseparably linked with the activity of the lipotropic agents. These findings obtained in many laboratories have greatly increased our knowledge of fat metabolism and its interrelationship with that of the other food materials.

Clinical Applications.

We have seen that choline (or its precursors) may exhibit dramatically favourable effects on the lesions produced in experimental animals *by a deficiency of choline in the diet*. We may expect that lack of choline in the human diet will produce similar disturbances but we have no proof, as yet, that this is so. If this point is established for the human subject we would then expect that choline would be an effective agent in the treatment of choline deficiency in human patients. There is little evidence that choline is lacking in what is at present considered an adequate diet. In countries where the protein intake is low a diminished intake of methionine might result in choline deficiency.

Apart from a deficiency of choline or its precursors in the diet there are several sets of conditions which might produce the signs of choline lack. The first is interference with protein digestion in the small intestine. There are a number of clinical abnormalities which may interfere with the normal breakdown of protein and in these the clinician should be on the watch for the signs of choline deficiency. We have seen that choline deficiency may be produced by this mechanism in experimental animals. Another possibility,

for which there is, at present, no experimental evidence, is that clinical abnormalities may exist in which there is interference with the methylation of amino-ethyl-alcohol or with some phase of the action of choline in the tissues.

I am greatly indebted to my colleague Professor C. C. Lucas for his help in collecting some of the data on which this lecture has been based.

References.

- (1) Banting, F. G., Best, C. H., Collip, J. S., Macleod, J. J. R. and Noble, E. C.: *Trans. Roy. Soc. Canada*, **16**, 27 (1922). — (2) Bouckaert, J. P. (quoted by Drury, D. R. (6)). — (3) Drury, D. R.: *Am. J. Physiol.* **131**, 536 (1940). — (4) Stetten, D., Jr. and Klein, B. V.: *J. Biol. Chem.* **162**, 377 (1946). — (5) Barrett, H. M., Best, C. H. and Ridout, J. H.: *J. Physiol.* **93**, 367 (1938). — (6) Stetten, D., Jr. and Salcedo, J., Jr.: *J. Biol. Chem.* **156**, 27 (1944). — (7) Weil, R. and Stetten, D., Jr.: *J. Biol. Chem.* **168**, 129 (1947). — (8) Campbell, J.: *Am. J. Physiol.* **147**, 742 (1946). — (9) Best, C. H. and Huntsman, M. E.: *J. Physiol.* **75**, 405 (1932). — (10) Best, C. H. and Huntsman, M. E.: *J. Physiol.* **83**, 255 (1935). — (11) Channon, H. J. and Wilkinson, H.: *Biochem. J.*, **29**, 350 (1935). — (12) Tucker, H. F. and Eckstein, H. C.: *J. Biol. Chem.* **121**, 479 (1937). — (13) Gavin, G. and McHenry, E. W.: *J. Biol. Chem.* **139**, 485 (1941). — (14) du Vigneaud, V.: *Harvey Lectures, Series 38*, 39 (1943). — (15) Welch, A. D. and Landau, R. L.: *J. Biol. Chem.*, **144**, 581 (1942). — (16) Stetten, D., Jr.: *J. Biol. Chem.* **140**, 143, (1941). — (17) McArthur, C. S.: *Science*, **104**, 222 (1946). — (18) Perlman, I. and Chaikoff, I. L.: *J. Biol. Chem.* **127**, 211 (1939). — (19) Boxer, G. E. and Stetten, D., Jr.: *J. Biol. Chem.* **153**, 617 (1944). — (20) Folch, J. and Woolley, D. W.: *J. Biol. Chem.* **142**, 963 (1942). — (21) Best, C. H., Lucas, C. C., Patterson, J. M. and Ridout, J. H.: *Science*, **103**, 12 (1946). — (22) Handler, P.: *J. Nutrition*, **31**, 621 (1946). — (23) Beveridge, J. M. R., Lucas, C. C. and O'Grady, M. K.: *J. Biol. Chem.* **160**, 505 (1945). — (24) Mulford, D. J. and Griffith, W. H.: *J. Nutrition*, **23**, 91 (1942). — (25) Handler, P.: *J. Biol. Chem.* **149**, 291 (1943). — (26) Rose, C. S., Machella, T. E. and György, P.: *Proc. Soc. Exp. Biol. Med.* **64**, 352 (1947). — (27) Griffith, W. H. and Wade, N. J.: *J. Biol. Chem.* **131**, 567 (1939). — (28) Christensen, K.: *Arch. Path.* **34**, 633 (1942). — (29) György, P. and Goldblatt, H.: *J. Exp. Med.* **72**, 1 (1940). — (30) Griffith, W. H.: *Biol. Symp.* **5**, 193 (1941). — (31) Dessau, F. I. and Oleson, J. J.: *Proc. Soc. Exp. Biol. Med.* **64**, 278 (1947). — (32) Patterson, J. M., Keevil, N. B. and McHenry, E. W.: *J. Biol. Chem.* **153**, 489 (1944). — (33) Chaikoff, I. L., Connor, C. L. and Biskind, G. R.: *Am. J. Path.* **14**, 101 (1938). — (34) György, P. and Goldblatt, H.: *Proc. Soc. Exp. Biol. Med.* **46**, 492 (1941). — (35) Lillie, R. D., Daft, F. S. and Sebrell, W. H.: *Public Health Reports*,

56, 1255 (1941). — (36) Blumberg, H. and McCollum, E. V.: *Science*, 93, 598 (1941). — (37) Webster, G.: *J. Clin. Investig.* 20, 440 (1941). — (38) Copeland, D. H. and Salmon, W. D.: *Am. J. Path.* 22, 1059 (1946). — (39) Engel, R. W., Copeland, D. H. and Salmon, W. D.: *Trans. New York Acad. Sci., Series II* 9, 89 (1947). — (40) Ralli, E. P., Rubin, S. H. and Present, C. H.: *Am. J. Physiol.* 122, 43 (1938). — (41) Montgomery, M. L., Entenman, C. and Chaikoff, I. L.: *J. Biol. Chem.*, 128, 387 (1939). — (42) Chaikoff, I. L., Entenman, C. and Montgomery, M. L.: *J. biol. Chem.* 168, 177 (1947). — (43) Best, C. H.: *Science*, 103, 207 (1946).

From Vestfold Fylkessykehus, Med. Depart. Tønsberg (Norway).
(Chief Physician: Dr. Med. A. Jervell.)

The Relation of Hypophysis to Carbohydrate and Basal Metabolism.

Cured Diabetes Mellitus and Hypothyroidism in Acromegalia.

(A Survey and a Case-record.)¹

By

HALVOR VERMUND.

(Submitted for publication November 7, 1947.)

Numerous clinical observations and experimental investigations have proved that the hypophysis is closely related to carbohydrate metabolism. It is thus a well-known fact that in illnesses where excessive function of the anterior pituitary is presumed to be present, diabetes often results. This so-called hypophyseal diabetes has been the subject of repeated thorough study, and the question as to the origin of the disease has been given special attention. The regulating influence of the anterior pituitary on the functions of the thyroid gland and through the latter on the general metabolism has been elucidated by numerous experiments on animals, and the question under discussion is whether the hypophysis plays any part in the development of hypothyroidism in man.

I.

Survey of the Relation of Hypophysis to Carbohydrate Metabolism.

a. Experimental Investigations.

The unitarian pancreatogenic diabetes-theory was based on the classical investigations of V. Mering and Minkowski (1), Allen (2), Banting, Best and associates (3), and from anatomico-patho-

¹ Read as a paper in condensed form at the annual meeting of the Norwegian Internal Medical Association on May 26, 1946.

logical point of view this theory was supported by Heiberg (4), Weichselbaum (5) and others. However, in many cases of diabetes there was found no typical histological changes in the pancreatic islets. (Warren (6), Allen (7).) Furthermore, clinical experiences went to show that other endocrine organs must have played a part in the development of certain forms of diabetes, studied under the name of extrainsular diabetes, the cause of which was supposed to be hormonal disturbances first and foremost of the hypophysis, next of the adrenals and other endocrine glands.

English and American physiologists have in recent years carried out extensive experiments on animals by which light has been thrown on the influence of the pituitary and adrenal glands on carbohydrate metabolism. More than 2,000 papers relating to this subject have been published during the past 20 years. Housay and associates (8, 9) have pointed out that the diabetes in pancreatectomized dogs was considerably improved and might even disappear after removal of the pituitary. Without insulin these animals could utilize considerable amounts of carbohydrates, which was shown by an increase in the respiratory quotient. If the animals were given enough food, the storage of glycogen in the liver and muscles proceeded normally, and hyperglycemia, glucosuria and acidosis disappeared. Numerous reports from other laboratories confirmed the correctness of these investigations, and it was thereby proved that the decreased carbohydrate combustion in pancreatectomized dogs could not wholly be attributed to insulin deficiency. (Houssay (10).) The pituitary somehow or other contributed to the onset of the diabetes.

By numerous experiments it has been proved that in the anterior pituitary certain substances are formed, which influence carbohydrate metabolism. There is some difference of opinion concerning these substances, but researches in recent years have, at any rate, proved the existence of two anterior pituitary hormones, regulating carbohydrate metabolism, namely the *diabetogenic* and *insulin-antagonistic or glycotropic* hormone.

Pancreatectomized dogs usually died of diabetes in the course of a few days if no insulin was given. But they lived for several months without insulin when the pituitary was removed. If these dogs without pancreas and pituitary were injected with anterior pituitary extract, there came an increase in the blood sugar concentration with consequent glucosuria, and the glucose tolerance decreased. Similarly, by injection of anterior pituitary extract

hyperglycemia could be produced in normal, healthy, non-operated animals. Borchardt (11) had already in 1908 noted transient hyperglycemia after injection of anterior pituitary extract. Evans et al. (12) in 1932 treated normal dogs with the growth hormone of the ant. pituitary and got as a result a disease similar to diabetes. Young (13) in 1937 discovered that daily intraperitoneal injections for a few weeks of large and steadily increasing doses of fresh anterior pituitary extract in dogs gave rise to diabetes with polydipsia, polyuria, hyperglycemia, glucosuria, acidosis, hyperlipemia and increased N-secretion in the urine. About the second day there was seen a temporary rise in the blood sugar concentration. After this there came a refractory stage of a few day's duration before the final diabetic condition set in, and this diabetes persisted even after the injections had been discontinued. In these animals Campbell and Best (14) found degenerative changes in the pancreatic islets and decreased insulin content in the pancreas. This diabetes differed from that found in dogs with extirpated pancreas inasmuch as the animals increased in weight during the whole of the period in which they were being injected (effect of the growth hormone?). The glycogen of the liver is also kept normal for a longer time in spite of abundant sugar secretion in the urine, than in the pancreatectomized animals. On the other hand, if insulin was injected simultaneously with the anterior pituitary extract, no signs of diabetes appeared and no changes of the pancreatic islets could be found.

The effect of the anterior pituitary extract of increasing the blood sugar has also been demonstrated in man. Lassen and Hansen (7) produced after the injection of alkaline anterior pituitary extract in doses equivalent to $2\frac{1}{2}$ grams of fresh gland tissue a rise of the blood sugar concentration both in the normal and the diabetic organism, accompanied by a marked increase of ammonia secretion in the urine. If the diet contained a small amount of protein and no carbohydrates, the effect was uncertain. The effect was most evident with a moderate amount of proteins and carbohydrates in the diet. It was also demonstrated that injection of the ant. pituitary extract could probably produce a temporary lowering of the sugar threshold of the kidneys in normal individuals.

The diabetogenic hormone has no effect on rats, mice and guinea-pigs, probably because these animals are able to develop an augmentation of their insulin-producing tissue. The amount of

endogenic insulin, together with other active factors, determines the mode of action of the diabetogenic hormone. If, therefore, the pancreas and pituitary are removed from these animals, there is seen a distinct diabetogenic effect of the anterior pituitary extract also on them. It seems as if these animals, by increasing their production of insulin, can neutralize the diabetogenic hormone, which proves that there is also an antagonism between the diabetogenic hormone and insulin.

Such an antagonism is clearly present between insulin and the second anterior pituitary hormone, affecting carbohydrate metabolism, namely the so-called glycotropic hormone. Houssay and Magenta (15) found that hypophysectomized animals were very sensitive to insulin. Hypoglycemic cramps could be caused by a much smaller dose of insulin than was necessary in normal animals. If these animals were treated with adequate doses of anterior pituitary extract, this supersensitivity decreased. (Houssay & Potic (16).) Young and associates in 1938 isolated the anti-insular principle from the thyreotropic, the lactogenic, the gonadotropic and the diabetogenic hormone, but its separation from the growth and the adrenocorticotrophic hormone could not be effected. Also in normal animals the glycotropic hormone was effective preventing hypoglycemia after injection of insulin (Young & Marks (17), Cope & Marks (18), Jensen & Grattan (19)). The glycotropic hormone maintains an abnormally high glycogen content in the liver and muscles during short periods of fasting, but in hyperglycemia it causes glycogenolysis. It had no effect on the fasting blood sugar in animals with extirpated pituitary, and thus differed from the diabetogenic hormone.

There has been much discussion about the specificity and mode of action of these anterior pituitary hormones. The published reports of the results of experiments are often conflicting, and it has been difficult to isolate the various active principles of the anterior pituitary from each other. In experiments carried out in order to throw light on the different active factors, it has frequently to be taken into consideration that many of them have an effect on other endocrine organs. Moreover, the various animals used in these experiments react differently to the same preparation. It has also been maintained that the substances obtained from the extracts of the pituitary are not identical with the hormones formed in the pituitary *intra vitam*. When these substances are extracted, they might split into several fractions,

each of them with definite effects, while the pituitary intra vitam only produces a few substances with more complex effects, acting upon different organs. (Christensen (20).) Some of the active principles, obtained in experiments, may be isolated on account of their different solubility in alcohol, acetone or certain salt solutions, or even by adsorption, ultrafiltration or other methods. In this way, it has been possible to separate the diabetogenic, glycotropic, thyreotropic, gonadotropic and lactogenic factors. (Collip (21).) On the other hand the attempts to separate the diabetogenic hormone from the growth and the adrenocorticotrophic hormones has not met with success. After experiments by Long and associates the diabetogenic hormone seems to be identical with the growth hormone and with the so-called ketogenic anterior pituitary hormone (Burn & Ling (22)), which produces as characteristic effect an increase of the acetone bodies of the blood and the urine.

The Relation of the Anterior Pituitary to the Adrenal Gland Cortex.

After extirpation of the pituitary there comes an atrophy of the adrenal cortex, but not of the marrow. The injection of anterior pituitary extract counteracts this atrophy by reason of its content of the adrenocorticotrophic principle. (Collin, Anderson & Thomson (23).) The adrenals have also effects on carbohydrate metabolism. It was well known from clinical practice that cases of tumors and hyperplastic processes in the adrenal cortex were accompanied by hyperglycemia and glucosuria. Among 55 patients with such tumors Lukens, Flippin and Thigpen (24) found glucosuria in 44 per cent. The disturbances in carbohydrate metabolism were explained as the result of an increased formation of sugar from protein and fat. It was maintained that the cause of hyperglycemia and glucosuria was a hyperfunction of the adrenal cortex. Numerous experimental investigations have also proved that the hormones of the adrenal cortex increase the blood sugar concentration and favour the accumulation of glycogen in the liver, while it has no effect on the muscle glycogen. Carbohydrate production in these cases proceeds at the expense of protein and fat. The effect of the diabetogenic anterior pituitary hormone is also, according to some experiments, dependent on increased formation of glucose from protein and fat. This so-called

focus is slowly reabsorbing while also miliary tubercles are disseminating in the lungs, and increases the hilum lymph-adenitis; moreover joints and hematogenic outspreading in the spleen (Fig. 3) and in the liver. At the 6th week the caseation also in the hilum lymphnodes appears.

Successively the extent of the former miliary tubercles of the lungs increases, tending to conglomerate into granulomatous, mostly caseous foci and giving place to a diffuse proliferation of the specific granuloma and following thickening of the interalveolar septa. Concomitantly also the other tubercular affected organs, *i. e.* spleen and lymph nodes, show a diffuse granulomatous involvement of the interstitial connective tissue.

The caseous change of the granuloma in the lung reaches its maximum after 8 or 9 weeks during which the sclerotic transformation of the tubercular process begins everywhere in the affected organs. This tendency assumes a particular significance in the liver where the connective tissue proliferation develops giving an anatomical picture similar to that of liver cirrhosis, namely consisting on the degeneration of the parenchymatous cells, a diffuse increase of the connective tissue starting from the portal spaces and sometimes from the centrum of the lobuli; a considerable splenomegaly with the characters of a diffuse fibrous inflammatory reaction and partially also as a secondary effect to the cirrhosis and correlated state of the blood in the portal district, as usually happens also in the spontaneous cirrhosis of the liver. The only organs that showed little or no tendency to the sclerotic transformation were the tracheobronchial lymph nodes, sometimes the cervical ones, that were found caseous prevalently in the latest stage of the disease.

Allergic Reaction.

The allergic reactivity evaluated, as above mentioned, on behalf both of the smallest sensibilizing tuberculin dose and from the superficial extent of the skin reaction by the constant anatuberculin test, showed a rather significative behaviour, desumed from the mostly uniform and linear results.

It was thus demonstrated that the *preallergic* period in the *subcutaneous* infected animals lasted about five weeks. Just at the end of the 5th week we could observe the first positive skin reaction, and successively a gradual slowly increasing reactivity

The liver also plays a part in this cooperation. Carbohydrate metabolism represents a dynamic balance between blood sugar formation in the liver and its utilisation by the body tissues. (Soskin (29).) In animals on which partial hepatectomy has been performed, the adrenal cortex hormones checks the fall of the blood sugar concentration, but after complete removal it has no effect. (Mann & Magath (30), Selye & Dosne (31).)

Epinephrectomized animals have the same insulinic hypersensitivity as those lacking the pituitary, and it has been maintained that the glycotropic hormone should also act by way of the adrenals. The results of the experiments relating to these matters are, however, conflicting, and the problems are as yet unsolved.

Other Hormones of the Anterior Pituitary Regulating Carbohydrate Metabolism.

The so-called glycostatic principle (Russell & Bennett (32)) was supposed to have the effect of keeping the muscle glycogen in fasting, hypophysectomized rats up to normal values, while the liver glycogen and the blood sugar fell to low values (Russell (33)). The pancreotropic hormone (Anselmino & Hoffmann (34)—Bierring (35)) was thought to lead to a slight fall of blood sugar level during a few hours following injection, and to produce an increase in the number and size of the pancreatic islets. It was claimed that this hormone produced the refractory stage, following injection of extracts of the anterior pituitary, containing the diabetogenic hormone. Another hormone, regulating carbohydrate metabolism, was claimed to produce low glycogen values in the liver, and still another hormone, found in extracts from the anterior pituitary, was supposed to increase the blood sugar level a few hours after the injection, while the diabetogenic hormone has no effect before at least some days have passed. But the investigations in this field are not finished, the results are partly conflicting and the existence of these hormones is not generally accepted.

b. Clinical Experiences Regarding the Effect of the Pituitary on Carbohydrate Metabolism.

The simultaneous occurrence of acromegalia and disturbances of carbohydrate metabolism is so frequent that it cannot be only incidental.

toms in 30 of the 63 patient who were treated. The improved patients exhibited a notable increase in carbohydrate tolerance. An insulin-resistant diabetic was treated with X-rays by Cannovo (cit. Lassen & Hansen (7)) with good results.

The Connection between the Pituitary and Carbohydrate Metabolism Elucidated by some Case Reports.

Lyall & Innes (62) report the case of a 27-year-old man, suffering from severe diabetes mellitus, which was in all respects a typical example of the insular form. The glucose tolerance curves were also similar to those found in this form. The patient was daily treated with 80 i. units of insulin, and the diabetes was controlled. By degrees the diabetic symptoms regressed, the insulin was withdrawn, and the patient took ordinary food and had urine free from sugar. The diabetes was cured. 10 months later X-ray examination revealed calcifications in a cystic hypophyseal tumor. It was presumed that this tumor was the cause of a decreased production of hormones. Before the development of the tumor the hormone-production of the anterior pituitary had hindered the secretion of endogenic insulin into the blood. When the tumor had destroyed the normal tissue of the anterior pituitary, this obstacle was removed, and the endogenic production of insulin was sufficient to maintain a normal blood sugar level.

Gripwall (51) has described a case of diabetes in acromegalia. The patient was a 56-year-old woman, in whom a typical acromegalia had developed during the course of a few years. The X-ray picture revealed a calcified nucleus, the size of a grain of corn, in the region of the sella turcica. The sella itself was normal. In this patient there developed signs of diabetes mellitus of the insular type with marked subjective symptoms, hyperglycemia, glucosuria and acetonuria. After adjustment with insulin, 36 int. units daily + diet, the urine was free from sugar and acid, and the fasting blood sugar was about 200 mg/100 cc. After a serious throat infection the urine became free from sugar, and the insulin could be withdrawn. The patient took ordinary food, and the blood sugar showed normal values. Her diabetes was cured. In order to explain the course of the disease, Gripwall supposed that the pituitary had been exposed to injury of a toxic or infectious nature in connection with the throat infection. Slight symptoms of diabetes insipidus simultaneously appeared, and the urine showed, according to the author's opinion, remarkably low values for the prolan concentration (less than 30 M. U. per litre). The acromegalia also showed a regressive tendency. Gripwall discusses to what extent the primary disturbance in the carbohydrate metabolism is caused by a hyperfunction of the pituitary or is a consequence of insufficiency in the insular apparatus or is due to both causes. He concludes that it is not possible to ascertain this, even if the glucose tolerance tests carried out some time after the diabetes was cured, seemed to indicate intact endocrine production in the insular tissue of the pancreas.

Oppenheimer (53) has described a case of acromegalia with diabetes of the insular type, ending in diabetic coma. A 35-year-old woman, who half a year ago had not showed any signs of disturbances in carbohydrate metabolism, was on admission in diabetic coma, on which the administration of insulin had no noticeable effect. The patient, however, recovered, and 5 weeks later she had no symptoms of diabetes. The glucose tolerance curves showed normal values, except for a prolonged reactive hyperglycemia, which was considered to be the result of good function of the insular tissue. Oppenheimer interpreted the patient's diabetes as being the result of disturbances in the production of pituitary hormones.

Kotte & Vonderahe (63) described a 40-year-old man, who had suffered from diabetes mellitus, which was controlled by insulin. He had moreover tuberculosis of both lungs with tubercular bacilli in the sputum. 2 months before admission he got severe headaches, vomiting, languor and loss of weight and was at last exhausted, half unconscious and perspiring. He had a temperature about 39° C, but on examination in the hospital the urine was free from sugar and acid. The patient died a short time after admission, and the blood sugar concentration was not investigated until some hours after death. It was then 31 mg/100 cc. Post mortem examination revealed necrosis of the anterior pituitary with a small cellular reaction and lack of conjunctive reactive tissue, which gave the impression of recent anatomo-pathological changes. The authors point out the resemblance of this case report to the hypoglycemic attacks in hypophysectomized animals, especially frequent in fasting, and the case was described as the *Houssay phenomenon in man*. In man, where the disease has caused rapid destruction of the anterior pituitary, low blood sugar level and symptoms resembling those found in hypoglycemic crises in hypophysectomized animals are noted and death follows before the typical Simmond's syndrome becomes manifest. The authors explained the clinical picture in this patient as the result of greatly decreased function of the anterior pituitary. On account of deficiency in the supply of nourishment during an intercurrent illness, the store of glycogen is used up, the infection further overworks the organs, giving rise to hypoglycemia similar to what is seen in animals with extirpated pituitary during fasting.

From physiological experiments we know the importance of the liver in carbohydrate metabolism. (Mann and Magath (30).) From clinical practice some cases also are reported of hypoglycemia in diabetes mell., where pathological conditions in the liver have been thought to play a part. (Rosendahl (64).) Joslin (65) states that the most frequent cause of hypoglycemia in diabetes is emaciation on account of insufficiency of diet. Spontaneous remission of diabetes which was supposed to be of hepatogenic origin, is reported by Motzfeldt (66).

Diseases with hypofunction of the pituitary are usually accompanied by increased carbohydrate tolerance and seldom by

gluconeogenesis, however, decreases after extirpation of the pituitary, while after injection of adrenal cortex hormone in hypophysectomized animals the gluconeogenesis increases. For this reason investigations were made to discover if the effect of the anterior pituitary on carbohydrate metabolism was conveyed by way of the adrenal cortex.

Viale's experiments (25) were of special interest in this connection. He pointed out that glucosuria after total or partial pancreatectomy on dogs decreased when the adrenals were extirpated. Epinephrectomy thus had a favourable effect on pancreatogenic diabetes similar to that of hypophysectomy. This effect is only obtained after removal of the adrenal cortex, not after demedullation or denervation of the adrenals. By administration of adrenal cortex hormone to pancreatectomized and epinephrectomized animals it was possible to produce hyperglycemia and glycosuria, and on large doses the values of the urine sugar excreted in 24 hours might exceed those found in pancreatectomized animals before the epinephrectomy. The adrenal cortex hormones thus have a diabetogenic affect.

Recent investigations have proved that the diabetogenic anterior pituitary hormone has to act together with the adrenal cortex to produce its full effect on carbohydrate metabolism. (Lundberg (26).) Injection of the diabetogenic anterior pituitary hormone has no effect on the blood sugar level, and there is no increase of the glycogen reserves of the liver in animals in which epinephrectomy has been performed. However, the glycogen content in the muscles rises. Thus it seems as if the regulation of the muscle glycogen is directly connected with the anterior pituitary, while the liver glycogen and the blood sugar are regulated by way of the adrenal cortex.

In pancreatectomized and epinephrectomized rats the anterior pituitary hormone, which was fully active in animals lacking the pituitary and the pancreas, did not cause glucosuria. If these animals, however, were given a certain amount of adrenal cortex hormone, there occurred abundant glucosuria after injection of ant. pituitary extract. From this Russel and Long (citation: Christensen (20)) came to the conclusion that a certain amount of adrenal cortex hormone was necessary to enable the pituitary factor to have any effect. It was evident that there is a synergism between the diabetogenic ant. pituitary hormone and the adrenal cortex hormones. (Long & Katzin (27) & Fry (28).)

but on a smaller scale, and the complete picture of myxedema is not seen. The basal metabolic rate is 15—25 per cent below normal, which is the same as seen in Simmond's disease. If, however, the thyroid is removed or damaged by disease, the basal metabolic rate falls to a new and lower level and adjusts itself to fairly constant values of about 60 per cent of the normal. This corresponds to the metabolic values found in complete athyreosis, which is accompanied by marked clinical signs of myxedema. It has been maintained that this is the lowest metabolic rate possible for the subsistence of life in not-hibernating mammals (Houssay (73)).

But it has also been maintained that after removal of the pituitary there is a greater decrease of the basal metabolic rate than after thyroidectomy, possibly because not only the thyroid gland, but also the adrenal cortex undergoes regressive changes (Kemp & Okkels (74)). In clinical practice hyperglycemia has been observed rather frequently in patients with hyperthyroidism, whereas in myxedema it is extremely rare. Joslin & Lahey (75) found glucosuria in primary hyperthyroidism in 38.6 per cent (228 cases), in secondary hyperthyroidism in 27.7 per cent (83 cases). Some of these patients had a transient disturbance of carbohydrate metabolism and were cured after subtotal thyroidectomy. After operation and cure of the hyperthyroidism the disturbance of carbohydrate metabolism returned to normal. Every patient with hyperthyroidism and disturbed carbohydrate metabolism cannot be classified as diabetic. Joslin raised the standard for a diagnosis of diabetes in hyperthyroidism to a blood sugar of 150 mg/100 cc fasting or 200 mg/100 cc or more after meals in addition to glucosuria. After this limitation true diabetes was seen in 2.5 per cent of the total cases of primary hyperthyroidism and in 4.3 per cent of secondary hyperthyroidism.

Hyperglycemia in hyperthyroidism is the result of secretion of adrenalin under the influence of the thyroid hormone through lysis of the liver glycogen.

The effect of thiouracil and similar substances in hyperthyroidism seems to be dependent on the action of the pituitary, and anatomo-pathological changes are observed in the cells of the anterior pituitary in experiments with similar substances. (MacKenzie, Mc. Collum and associates — cit. Rune Frisk (76).)

Numerous investigations have been made to ascertain whether certain forms of myxedema are caused by deficient secretion

diabetes. Maranon & Morros (38) found among 115 cases of dystrophia adiposo-genitalis following hypofunction of the pituitary only 2 cases of diabetes mellitus. But hypofunction of the anterior pituitary does not always exclude diabetes mellitus. (John (67), Allan & Rowntree (68), Feldman, Roberts, Susselman & Lipetz (69).)

II.

The Effect of Pituitary on Basal Metabolism.

The pituitary is superior to the thyroid and governs its activity by aid of the thyreotropic hormone (Aron (70), Loeb et al. (71)). After hypophysectomy regressive changes appear in the thyroid. The thyroid has also an effect on the vegetative centres of the mid-brain and other endocrine organs, and is closely in tune with them. This mutual dependence is necessary for an orderly metabolism. The thyreotropic hormone in the anterior pituitary stimulates the hormonal secretion of the thyroid. This effect could successfully be demonstrated in young guinea-pigs. After injection of anterior pituitary extract the cells of the thyroid follicles swell out, the vesicles become smaller, the colloid is vacuolised, and proliferative changes in the cells take place. The result is increased secretion of thyroid hormone in the blood and a higher basal metabolic rate. This reaction is so characteristic that it is used as a test for the thyreotropic hormone.

The thyreotropic hormone has been isolated from the other pituitary hormones and has been found in watery extracts from the anterior pituitary. After injection of the hormone, besides an increase of the basal metabolic rate, also exophthalmus, tachycardia and increased irritability can be observed, and the thyroid gland increases in size, while the iodine content diminishes. In thyroidectomized animals no such symptoms are seen, which proves that the effect occurs by way of the thyroid gland. If the hormone acts for a longer time, the stimulating effect on the thyroid is diminished, which should be due to the so-called antihormones with antagonistic effect on the thyreotropic hormone. But many authors deny the existence of these antihormones.

After hypophysectomy the basal metabolic rate falls, and adjusts itself after a time to a new and lower level. But it is some dissension between the various authors how much it falls. Means (72) states that the thyroid still functions after hypophysectomy,

Case-record.

An unmarried woman (serial No. 2302/46 G. A.), aged 46, was admitted to the hospital for the first time on Oct. 20, 1945, under the diagnosis of diabetes mellitus and imminent coma.

The family history revealed no known cases of diabetes or other illnesses of interest. In 1917 she was treated for syphilis and went through an additional antisypilitic treatment in connection with child-birth in 1928. She gave birth to a fully developed child, who later has shown no signs of congenital syphilis. Otherwise the patient had been quite fit until in May 1945 she consulted the doctor on account of obesity, developed during the course of 4—5 weeks. Her clothes had become too tight and her feet so large that although she had been using previously ladies' shoes No. 39, she now needed mens' shoes No. 43. Her hands also had increased in size, and rings previously worn, became too small. Her features became so altered that people did not recognize her in the street.

At the end of August 1945 the patient suffered from extreme thirst and polyuria and voided at least 3—4 liters of urine every night. She also suffered from pruritus vulvae. After a while there came increasing languor and fatigue with loss of weight despite a hearty appetite. Besides, she was much troubled by cramps in the legs and had paresthesiae and pricking pains in the lower extremities when walking. There were no gastric alterations, but she suffered from constipation. Menopause occurred 5 years before admission. Libido sexualis had noticeably diminished during the summer 1945.

The present status was found to be as follows:

The patient has a typical acromegalic appearance with rough, plump features. The nose was thick and large with broad nostrils. The chin, cheek-bones and the region round the eye-brows were strongly developed and prominent. The whole head seemed large with prominent protuberantia occ. ext. The ears were large, the aural meatus narrow and her hearing was impaired. Her lower lip was thick and slightly everted, the tongue broad and large, and the mucous membrane of the mouth seemed on the whole rough and thick. In the lower jaw there were large spaces between each tooth (»railing teeth»). She had deep and extensive caries. Her bearing was stooping, and in the thorax was found increased kyphosis and moderate scoliosis. Sternum, clavicle and ribs seemed thickened. Her hands and feet were enormously enlarged with plump fingers and toes. The extremities were long and large, especially in the distal parts. The skin was rough and had large folds and wrinkles in the face. Diffuse, irregular pigmentations and small fibromas were found over the trunk and extremities. Secretion of sweat seemed diminished. Her hair was coarse and straight, but fairly sparse and the eye-brown bushy. There was no pallor or icterus. Weight 65 kg. Height 157 cm. She was sleepy and gave the impression of languor and fatigue. Temp. 37.°4 C., pulse 76, regular. Respiration 16, deep (Kussmaul's type) with marked aceton odour. Blood pressure 140/90.

There was slight edema of the lower parts of the legs. The thyroid gland was not enlarged. The liver could not be felt at the costal margin, and nothing pathological was to be observed on physical examination of the inner organs. The tendon-reflexes of the lower extremities were absent, and there was marked tenderness on palpation of the calves. No disturbances of motility and sensibility. Plantar reflexes normal on both sides.

Urine was acid, light yellow, clear, specific gravity 1028. The reaction of Benedict ++. Rothera +++++, Gerhard +, Heller +. In the sediment were found many short, broad coma-cylinders.

On admission the blood-sugar concentration was over 385 mg/100 cc. 4 hours later 396 mg/100 cc (Hagedorn-Norman-Jensen). Plasma-bicarbonates: 9.5 m.eqv./l.

From these investigations it was clear that the patient, besides the signs of acromegalia and polyneuritis, showed the clinical picture of imminent diabetic coma, for which reason there was immediately instituted treatment with rapidly-acting insulin, in all 80 I. U. the same day with 1,200 cc. 2.6 % solution of soda bicarbonate intravenously and 20 grams soda bicarbonate powder per os.

After this she regained consciousness and was the next morning considerably better with normal respiration. The concentration of the plasma bicarbonates had risen to normal values.

Laboratory investigations:

Blood: SR 43 mm/1 hour.

Hemoglobin 95 % (Sicca)

Red blood count 4.13 mill./mm³

White blood count 10,300 per mm³

Colour index 1.16

Meinicke's reaction: Negative.

Calcium in serum: 10.3 mg/100 cc

Phosphorus in serum: 3.6 mg/100 cc

Soda bicarbonate in plasma (Oct. 23.): 27.3 m.eqv./l.

(The patient had the 2nd day after admission received 60 grams of bicarbonate of soda per os. Later this was withdrawn).

Basal metabolic rate on the 4th day after admission: 126 %.

The blood/urine sugar relation was as shown in the curve (see Fig. 1).

The first 12 days after admission the fasting blood sugar showed a marked varying tendency with values between 140 and 350 mg/100 cc. The amount of sugar in urine varied between 70 and 186 grams per 24 hours. Concentration of sugar in the 24-hour urine was between 5.8 and 7.8 %, and the spec. gravity of the urine varied between 1035 and 1040. The average diuresis was between 1,000 and 2,000 cc with a maximum some days of 3,000 cc. The patient daily received large doses of insulin — between 72 and 112 I. U. per 24 hours. She was during this time on a diet with 2,230 calories (189 g carbohydrates, 89 g proteins and 114 g fat). After some days the urine reaction of

GLUCOSE TOLERANCE CURVES

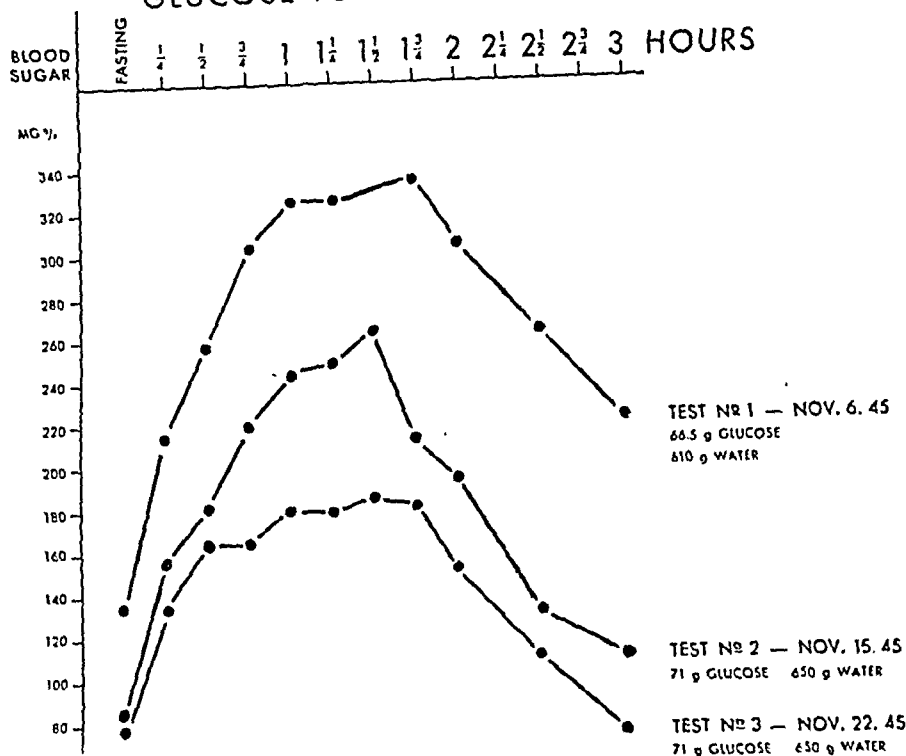


Fig. 2.

peated sugar tolerance tests performed in this period gave a good illustration of the course of the illness (see fig. 2).

The first curve shows the result of the glucose tolerance test performed 4 days after the hypoglycemic attack (Nov. 6, 45) and is typical of ordinary diabetes. The second test was carried out one week later (Nov. 11.), and there was now only an unimportant deviation from a normal glucose tolerance curve. Nearly 3 weeks after the hypoglycemic attack the third test showed a completely normal curve. (Nov. 22.)

The patient's diabetes, which one month previously had been threatening with regular diabetic coma, had to be regarded as cured.

In the 2 years which have elapsed since that time, the patient has shown neither subjective nor objective symptoms of diabetes.

The clinical diagnosis of acromegalia was confirmed by X-ray examination (Oct. 23, 45). The roentgenogram of the skull showed considerable changes in the sella turcica. Processus clinodei ant. were coarse, and sella turcica considerably extended downwards and backwards. Proc. clin. post. had entirely disappeared, probably as a result of pressure. From proc. clin. ant. toward dorsum sellae was seen a narrow, striped calcium shadow having a bow-like curve. The density of the



Fig. 3.

skull was somewhat increased, and the protuberantia occipitalis ext. enlarged. The sinus frontalis as well as the pneumatic cavities of the mastoidal region seemed to have increased in extension.

Roentgen diagnosis: Hypophyseal tumor (see Fig. 3).

X-ray examination of the hand revealed the changes characteristic of acromegalia, with coarse, plump phalanges and large, broad processus unguiculares. X-ray picture of the columna showed spondylosis deformans incipiens and scoliosis. Roentgen examination of the thorax: Parenchyma of the lungs clear, normal diaphragmatic movements, and the heart was of normal configuration and not enlarged (transversal measurement 13.5 cm, the internal diameter of the thorax 29 cm).

Examination of eyes (Tonjum): Transparent mediums. Visual capacity: 5/50, after correction by + 5: normal 5/5). *Perimetry*: Normal limits of field of vision and no scotomy.

Ophtalmoscopy: Normal fundus.

During the stay at the hospital the treatment of the polyneuritis of the lower extremities was carried out with vitamin B₁. For the last 14 days of hospital observation she was up and about and was

discharged in good health, the total time of internment being 5 weeks.

The patient was advised to come to control at the medical policlinic station, but she did not, because she had in the meantime been admitted to another hospital on account of languor, anorexia, nausea, vomiting, headache and attacks of loss of consciousness with tremors, during which she fell down on the floor. She had altogether 3 such short attacks, which evidently were not accompanied by convulsions, paralysis or diplopia, but were followed by repeated attacks of vomiting.

Three months after the date of the first admission in our hospital she was readmitted, this time under the diagnosis of pneumonia. In the course of a couple of months she developed a marked languor, so that she had difficulties in being up and about. She became dull and slow and by degrees indolent and disinterested, did not give attention to what was going on around her, had a bad memory, felt the cold easily and mostly sat dozing by the stove. She fell asleep as soon as she sat down on a chair at all hours. Her voice was hoarse and sounded like a man's voice. Her skin was dry, cold and peeling, and there was considerable loss of hair. Her nails were thick and brittle and grew slowly. She never perspired. She suffered from headache, giddiness, noises in the ears, shortness of breath on exertion, but no angina pectoris. She easily got edema of the legs in the afternoon.

3 days before admission she got symptoms of an intercurrent air passage infection with slight fever, pain in the chest and cough with expectoration. Her appetite was bad, and she suffered from a light, uncharacteristic dyspepsia, and from a severe constipation. 4—5 days passed without defaecation, and it became necessary to use a laxative, which, however, had little effect. A couple of days before admission she got diarrhea.

On examination it was astonishing to see how great a change had occurred in the patient's appearance since the last admission. The acromegalic features were still present, but not so outstanding. Most remarkable was the obvious mental and physical asthenia. Her attention was difficult to keep and could not be held for a long time. Her power of perception was greatly reduced and the memory bad. The answers came slowly and hesitatingly in a hoarse, rough and rasping voice. Mostly she lay dozing, but was quite conscious and aware of time and place. Her skin was pale with slightly flushed cheeks. It was coarse and rough with a fine lamellous desquamation and felt cold and dry to the touch. It seemed thickened with large folds and wrinkles, but was not edematous. The chair of the head was thin, dry and brittle. The eye-brows were very sparse, and the hair of the axilla and pubic region absent.

The face itself seemed expressionless, with little facial play, apathetic and vacant, the wrinkles of the forehead were large and thick, the eyelids swollen and the orbits narrow. The lips were large, the tongue moist and clean, but with thickened mucous membrane. The whole figure was fat, heavy and round-shouldered with large hands and feet. Weight 65 kg. Height 157 cm.

The temperature kept slightly subfebrile the first days, but then fell below normal.

Pulse 80, regular. The following days there came an obvious bradycardia with a pulse frequency around 44.

Blood pressure 110/75.

The thyroid gland was of normal size. No rashes.

In the fauces was found a little mucous on the posterior pharyngeal, but the tonsils were normal. Over the lungs were heard some sibilant and rhonci, otherwise nothing pathological on routine physical examination.

Urinalysis gave no pathological findings.

Blood sedimentation 65 mm/1 hour.

Hemoglobin 85 per cent (Sicca).

Leucocytes 18,500.

Meinicke's reaction negative in blood and spinal fluid, and the spinal was normal on routine laboratory examination.

X-ray of pulmonary organs: Normal findings.

X-ray of the skull: Unchanged conditions since previous examination.

Ewald's test-meal: 150 grams aspirated, well digested. Free acid 58, total acidity 91.

Benzidine test in stools: Negative on repeated examinations.

Cholesterol estimation in plasma: 289 mg/100 cc.

Serum chloride: 96.4 m.eqv./l.

Alkali-reserve: 21.4 m.eqv./l.

Serum-proteins: Total protein: 6.35 per cent

Albumin: 3.94 " "

Globulin: 2.41 " "

A/G: 1.63

N. P. N: 28.02 mg/100 cc.

Electrocardiogram showed low voltage in 1st lead and flat T-waves in all leads.

Repeated basal metabolism tests showed obviously low values:

On March 4th: 63 per cent

" " 5th: 53 " "

" " 6th: 63 " "

" " 11th: 66 " "

New glucose tolerance tests were performed. One of the curves (March the 8th) was rather flat. The fasting blood sugar was low (57 mg/100 cc). After 68 grams of glucose per os the blood sugar level rose to 148 mg/100 cc 45 min. later. After 3 hours it was again down to 61 mg/100 cc. The other curves were normal (see Fig. 4).

The clinical picture together with the tests of the basal metabolic rate made it clear that the patient suffered from marked hypothyroidism. Therefore the treatment with thyroid gland extract was adopted with doses in the first days equivalent to 0.30 grams of normal gland and increasing up to 0.60 grams daily.

Under this treatment there was a progressive improvement in her

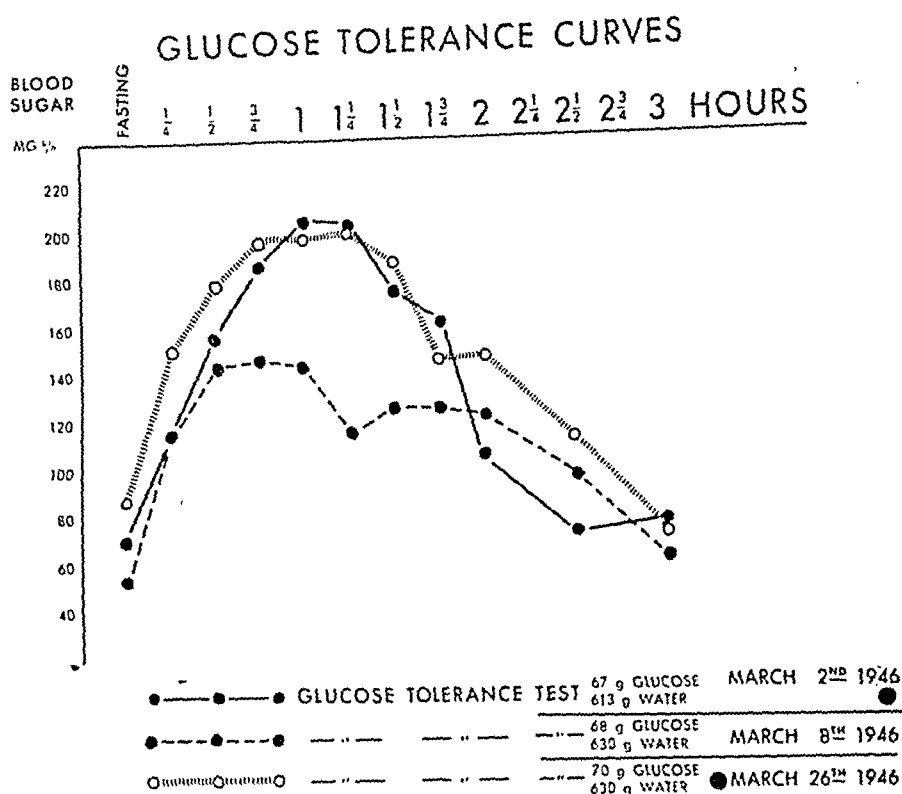


Fig. 4.

condition. Mentally she improved and became amiable and smiling, fond of joking, and giving quick and clear answers when questioned. Her voice became less rough and the speech normal. She was no longer dull and indolent. The appetite improved.

The basal metabolic rate increased to 92 per cent after a fortnight's treatment, and after 1 month in hospital the patient was discharged in good health.

The third admission was on May 20th 1946.

She had suffered somewhat from dyspnoea on exertion since her last stay, otherwise she felt quite well. The symptoms of hypothyroidism had completely disappeared, and the patient's acromegalia continued to show a regressive tendency. She stated that her hands and feet had become smaller. Rings that she previously had found too small were now easily worn, and she used No. 40 in ladies' shoes, while she before had used men's shoes No. 43.

She had been taking thyroid extract in doses equivalent to 0.60 grams normal gland daily.

On the usual clinical examination, regression of the symptoms of acromegalia was confirmed, and there were no clinical signs of hypothyreosis. Physical examination revealed nothing of interest. Urinalysis revealed no pathological findings.

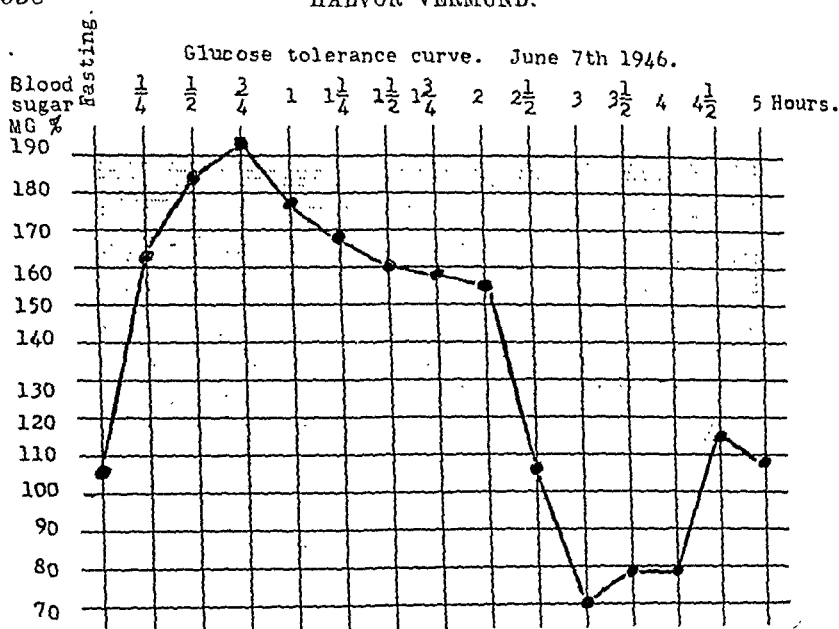


Fig. 5.

The thyreoid preparation was withdrawn as an experiment, after which the basal metabolic rate sank to 70 per cent, and the patient became more slow and sleepy. The fasting blood sugar, however, kept its normal values about 100 mg/100 cc. A glucose tolerance test on June 7th showed a normal curve with the usual post-alimentary hypoglycemia (see Fig. 5).

There was also carried out two insulin tolerance tests with 5 int. units insulin intravenously, the results being as indicated in Fig. 6. The first test (May 22.) was performed before the thyroid extract was withdrawn, the second (June 7.) at the time when the basal metabolic rate was about 70 per cent.

The first curve showed a fall in the blood sugar concentration from 98 mg/100 cc to 40 mg/100 cc after 25 min., the second from 108 mg/100 cc to 34 mg/100 cc after 30 min., with at the same time slight symptoms of hypoglycemia without cramps or loss of consciousness.

An adrenalin tolerance test on June 8th with 1 mg subcutaneously gave a slow blood sugar increase from 92 mg /100 cc to a maximum of 181 mg/100 cc after 1 hour 30 min. (see Fig. 7).

Other laboratory investigations:

Serum bilirubin: 9 (Meulengracht's method)

Takata: negative.

Serum-albumin: 4.09 per cent.

Serum-globulin: 2.56 per cent.

RELATION OF HYPOPHYSIS TO METABOLISM.
INSULIN TOLERANCE CURVE - MAY 22th 1946.

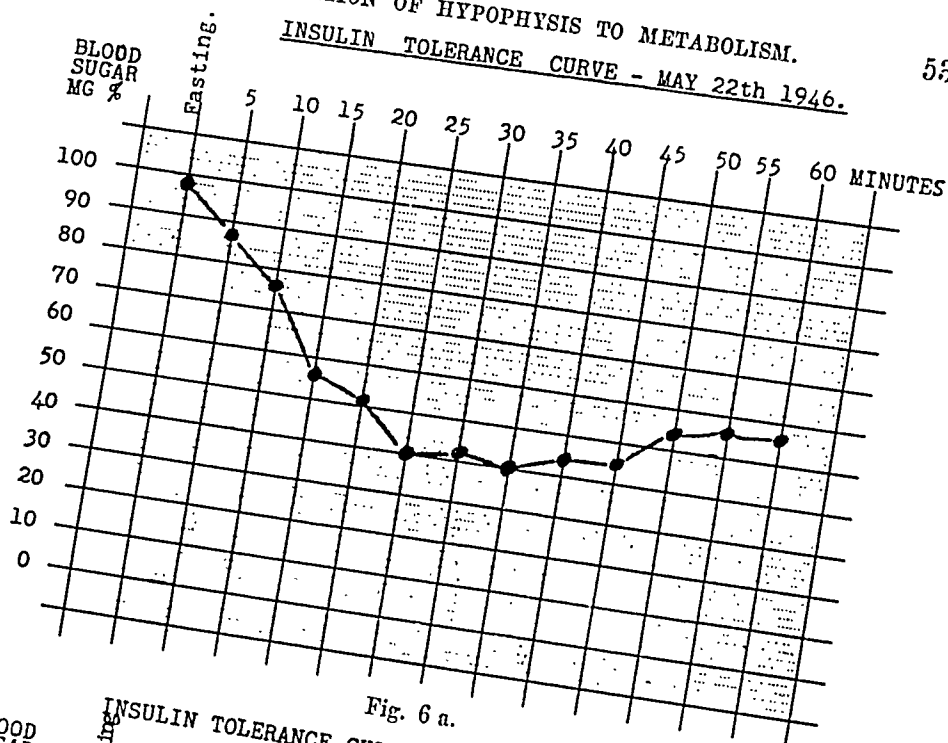


Fig. 6 a.

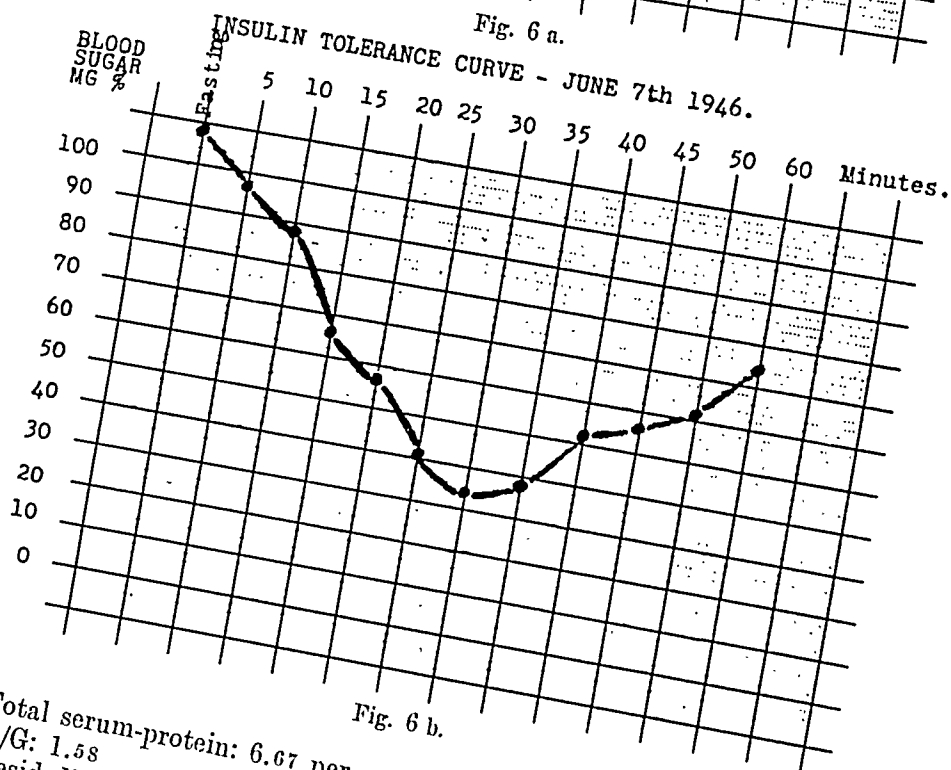
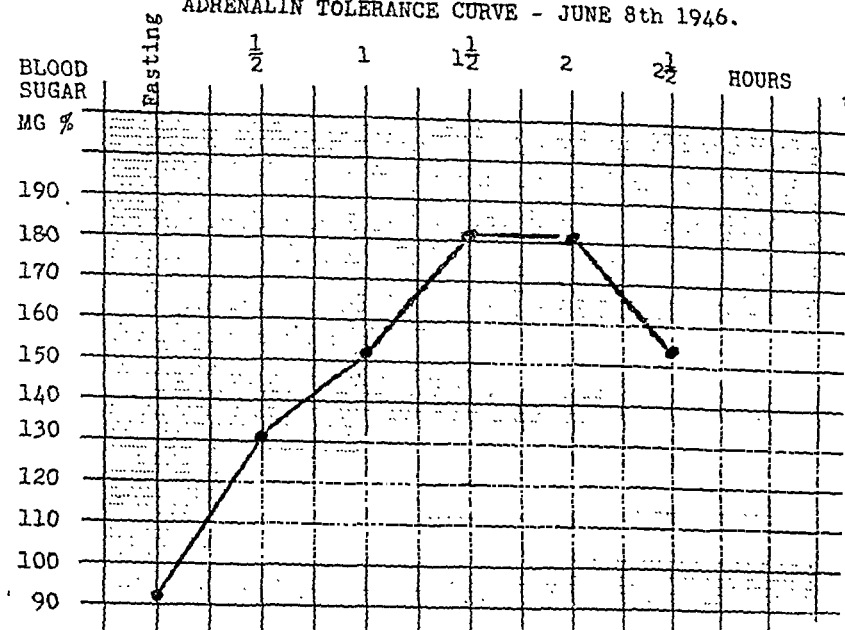


Fig. 6 b.

Total serum-protein: 6.67 per cent.
A/G: 1.58
Resid. N in serum: 34.7 mg/100 cc.
Cholesterol in plasma: 214 mg /100 cc.

FIG. 7.

ADRENALIN TOLERANCE CURVE - JUNE 8th 1946.



A hormone determination in the urine (Nygård & Co. Biological laboratory) have the following result:

Prolan A and B: 33 Mouse-units per litre.

Folliculin: $> 17 < 56$ Mouse-units per litre. (Closs).

According to comments from the biological laboratory's manager, P. Laland, B. Pharm., it appears that determination of the gonadotropic hormones shows low values, similar to those found in women at this age. The quantity of oestrogenic hormone is normal. He concludes »Unfortunately it is not possible on this investigation to give any information as to whether there are any signs of reduced secretion of gonadotropic hormones. The quantities normally precipitated, are so small that they are often difficult to find. Any further morbid reduction in the precipitation is not possible to ascertain.»

The patient was told to continue with the same doses of thyroid and was discharged in good condition.

She has later been controlled at the policlinic station up to November 1947. For the most time she has been in good health, but on the last examination some hypothyreotic signs had reappeared, because she had taken too small doses of the thyroid gland extract.

Discussions on the Case Reported.

Judging from previous clinical experiences and results attained by experimental investigations regarding the influence of the pituitary on the carbohydrate metabolism, there is reason to suppose that the diabetes in our case had arisen as a direct consequence of the disease of the pituitary. It is supposed that the disease in the beginning caused a hyperfunction of the ant. pituitary's gland tissue. Probably there has been an eosinophilic adenoma or some hyperplastic process of another nature in the anterior pituitary, with increased secretion of the growth hormone, resulting in acromegalia. During this period the patient had a moderate increase of the basal metabolic rate, which might point to increased production of the thyreotropic hormone. Experimental investigations have shown that the growth hormone in all probability is identical with or at least closely related to the diabetogenic and possibly to the adrenocorticotrophic hormone. Therefore, it may be supposed that the hypersecretion also included the hormones of the ant. pituitary, regulating carbohydrate metabolism.

The glycotropic hormone has an antagonistic effect on the endogenic insulin and the diabetogenic hormone causes at the same time an increased production of glucose at the expense of protein and fat, resulting in acidosis, probably in great part a consequence of the hypersecretion of the adrenal cortex. In this way we can explain the development of the disturbances in carbohydrate metabolism, identical with those seen in diabetes mellitus. It seems to us rather unnatural to draw a sharp line between pituitary and insular diabetes mellitus. In the ordinary forms of diabetes in acromegalia it must be assumed that the endogenic production of insulin is still sufficient to prevent serious disturbances in carbohydrate metabolism. The course is non-malignant, and there is little tendency to acidosis. The restricting influence of the anterior pituitary hormones on the endogenic insulin production is not complete, and the diabetogenic hormone is not produced in such quantities that it hinders the normal carbohydrate metabolism to any extent. The surplus carbohydrate-regulating pituitary hormones, however, causes more or less resistance to insulin. Where there is a considerable hypersecretion of the anterior pituitary hormones which influence

carbohydrate metabolism, the clinical picture changes and takes more the character of the insular diabetes, which is rather resistant to insulin, as was also seen from our case report. This resistance may be the only mark of distinction from ordinary insular diabetes.

It is still, however, an unsolved problem to what extent the insulin-resistant forms of ordinary diabetes without signs of pituitary disease are of hypophyseal origin.

In order to explain the progressive course of the illness in our patient we must suppose that the pituitary had become the seat of regressive changes with hypofunction of the anterior lobe as a consequence. The obvious decrease of the acromegalic signs points in this direction. We must suppose that the surplus of diabetogenic and glycotropic hormones is eliminated, and then the quantity of endogenic insulin can maintain normal conditions in carbohydrate metabolism.

It would have been of great interest to have ascertained whether our patient showed signs of pituitary hypofunction, similar to those produced in animals in experimental investigations by Houssay et al. Animals lacking pancreas and pituitary, have a tendency to hypoglycemia during fasting. It was supposed that our patient had an insufficient production of insulin, and if there also was a hypofunction of the ant. pituitary, we might expect hypoglycemia during fasting and a considerable supersensitivity to insulin. The symptoms in our patient between the first and second admission were similar to those produced by hypoglycemia. The fasting blood-sugar was also low during the second stay, but adjusted itself later on up to normal values after treatment with thyroid gland extract. After withdrawal of this medication we might have expected a fall in the fasting blood-sugar values, but no hypoglycemia was found. Neither did the glucose tolerance tests show any deviations from the normal, and the result of the insulin tolerance tests lay within the limits of the normal variation (confer the investigations of Meyer & Melzl (81)). The insulin tolerance tests in normal persons show great variations. After injection of 5 i. u. of insulin intravenously the blood sugar concentration falls 30—50 mg/100 cc in 2/3 of the cases during the first hour. But in some cases where carbohydrate metabolism was normal, the fall of the blood sugar concentration was up to 82 mg/100 cc. Himsworth's test has not been made.

The adrenalin tolerance test, carried out to detect any hypofunction of the adrenal cortex, showed only slight deviation from

a normal curve. In normal persons the rise of the blood sugar level should be at least 45 mg/100 cc above the initial value, and the curve should reach a maximum within one hour. (Broesameln (82), Heni (83).) In our case the maximum value of 89 mg/100 cc above the initial value was reached 1 hour 30 min. after the injection. This slight deviation could not give any indication of hypofunction of the adrenal cortex.

Neither gave the determination of gonadotropic ant. pituitary hormones in the urine any results pointing to hypofunction of the pituitary. From these investigations we were obliged to conclude that neither the pituitary nor the adrenal cortex showed any insufficient hormone production at the time when the tests were carried out. We also had to suppose that the function of the pancreatic islets was reestablished. Neither was there any reason to assume that some disease of the liver was the cause of the disturbances in carbohydrate metabolism.

The next question was whether reduced production of thyreotropic pituitary hormone could have produced the hypothyreosis in the patient. The exceedingly low basal metabolic values might possibly be assigned to hypofunction of the ant. pituitary at the time just before the treatment with thyreoid gland extract. At this time we might suppose the adrenal cortex to be in a state of hypofunction as a consequence of too little secretion of adrenocorticotrophic hormone from the ant. pituitary. It is at any rate difficult to explain basal metabolic values of 53 per cent on the basis of insufficient thyreoid function only. The investigations performed two months later after the treatment with thyreoid gland extract, however, revealed no pituitary hypofunction. Such a hypofunction might after all have been present earlier. A transitory withdrawal of the thyreoid extract could not reestablish the previous conditions, and a more protracted withdrawal was considered too risky for the patient.

Determination of the content of thyreotropic pituitary hormone in the blood of the patient could not be carried out in our country at that time.

Summary.

Report is made of a case where the patient — a 46-year-old woman — had signs of acromegalia, and the X-ray picture showed a tumor of the pituitary. Three months before admission the

patient had symptoms of diabetes mellitus, and on examination in the hospital she had considerable acidosis with imminent coma. She recovered after administration of soda bicarbonate and insulin, but the blood-sugar concentration was still high, and urinalysis revealed a considerable excretion of sugar in spite of large doses of insulin.

On the 12th day after admission signs of hypoglycemia suddenly appeared. Insulin and dietary restrictions were withdrawn, and after this time there was no hyperglycemia and no glucosuria. The glucose tolerance test gave in the beginning a typical diabetes-curve, later on a completely normal one, and the diabetes had to be considered as cured.

Then there developed an advanced hypothyroidism with extremely low basal metabolic rate, which, however, remitted after treatment with thyroid gland extract. The signs of acromegalia showed regression, and the patient has since been in good health and quite fit for work.

After a survey of the relation of the hypophysis to the carbohydrate and basal metabolism, based on studies of the literature, the author points out that the whole clinical picture, as reported above, may be interpreted as the result of disturbances in the hormonal production of the anterior pituitary. A transitory hyperfunction of the anterior pituitary is accompanied by increased production of the growth hormone, resulting in acromegalia, and by increased production of the diabetogenic and glycotropic hormone, the result of which is disturbance of the carbohydrate metabolism, resembling those seen in diabetes mellitus.

The hyperfunction is followed by hypofunction of the ant. lobe after which the acromegalia regressed, the diabetogenic and insulin-antagonistic effects of the anterior pituitary hormones disappeared, and the endogenic insulin production is now sufficient to re-establish normal conditions in the carbohydrate metabolism. This hypofunction was also supposed to be the origin of the patient's hypothyroidism on account of decreased production of thyrotropic hormone in the ant. pituitary.

Investigations made two months later when the basal metabolic rate had reached normal values after treatment with thyroid gland extract, could not, however, prove the correctness of this hypothesis, and consequently the problems presented by the recorded case are still open to discussion.

Bibliography.

1. Minkowski, O., v. Mering, J.: *Centralblatt Klin. Med.* 1889: 23: 393.
- 2. Allen, F. M.: *Journ. Americ. Med. Ass.* 1914: 63: 939. — 3. Banting, F. G., Best, C. H., Collip, J. B., MacLeod, J. J. R., Noble, E. C.: *Americ. Journ. Physiol.* 1922: 62: 162. — 4. Heiberg, K. A.: *Die Krankheiten des Pancreas.* Wiesbaden 1914: 238. — 5. Weichselbaum, A. cit. by Graham C.: *Brit. Encyclop. of Med. Practice.* London 1937: 3: 646. — 6. Warren, S. cit. by Grafe, E., Tropp, C.: *Handbuch d. inneren Med.* Edited by Mohr, L., Staehelin, R., v. Bergmann. 3. Aufl. 1944, Band 6 II: 513. — 7. Lassen, H. C. A., Hansen, L. (cit. Allen): *Hospitals-tidende* 1937: 80: 1145. — 8. Houssay B. A., Biasotti, A.: *Archiv f. d. gesamte Physiol.* 1931: 227: 239, 664. — 9. Houssay, B. A., Biasotti, A.: *Endocrinology* 1931: 15: 511. — 10. Houssay, B. A., Biasotti, A.: *Am. Journ. Med. Science.* 1937: 193: 581. — 11. Borchardt, L.: *Zeitschrift Klin. Med.* 1908: 66: 332. — 12. Evans, H. M., Meyer, K., Simpson, M. E., Reichert, F. L.: *Proceedings of the Soc. for Exp. Biol. and Med.* 1931—32: 29: 857. — 13. Young, F. G.: *The Lancet*, 1937: 2: 372. — 1936: 2: 297. — 14. Campbell, J., Best, C. H.: *The Lancet*, 1938: 1: 1944. — 15. Houssay, B. A., Magenta, M. A.: *Compt. rendus de la Soc. de Biol.* 1925: 92: 822. — 16. Houssay, B. A., Potick, D.: *Compt. rendus de la Soc. de Biol.* 1929: 101: 940. — 17. Young, F. G., Marks, H. P.: *Journal of Physiology* 1938: 93: 61. — 18. Cope, O., Marks, H. P.: *Journal of Physiology* 1935: 83: 157. 19. Jensen, H., Grattan, J. F.: *Americ. Journ. of Physiol.* 1940: 128: 270. — 20. Christensen, B. G.: *Nord. Medisin* 1942: 14: 1311. — 1386. — 21. Collip, J. B.: *Journ. Am. Med. Ass.* 1935: 104: 827, 916. — 22. Burn, J. H., Ling, H. W.: *Journ. of Physiol.* 1928: 65: 191. — 23. Collip, J. B., Anderson, E. M., Thomson, D. L.: *The Lancet* 1933: 2: 347. — 24. Lukens, F. D. W., Flippin, H. F., Thigpen, F. M.: *Am. Journ. of Med. Sciences* 1937: 193: 812. — 25. Viale, G.: *Klin. Wochenschrift* 1933: 1: 467. — 26. Lundberg, N. E.: *Nord. Medisin* 1940: 8: 1731. — 27. Long, C. N. H., Katzin, B.: *Proc. of the Soc. f. Exp. Biol. and Med.* 1938: 38: 516. — 28. Long, C. N. H., Katzin, B., Fry, E. G.: *Endocrinology* 1940: 26: 309. — 29. Soskin, S.: *Endocrinology* 1940: 26: 297. — 30. Mann, F. C., Magath, T. B.: *Am. Journ. of Physiol.* 1923: 65: 403. — 31. Selye, H., Dosne, C.: *Am. Journ. of Physiol.* 1940: 128: 729. — 32. Russell, J. A., Bennet, L. L.: *Am. Journ. of Physiol.* 1937: 118: 196. — 33. Russel, J. A.: *Proceedings of the Soc. f. Exp. Biol. and Med.* 1936: 34: 279. — 34. Anselmino, K. J., Herold L., Hoffmann, F.: *Klin. Wochenschrift* 1933: 12 II, 1245, 1436. — 35. Bierring, K.: *Acta Med. Scand.* 1936: Suppl. 78: 575. — 36. Davidoff, L. M.: *Endocrinology* 1926: 10: 461. — 37. Yater, W. M.: *Arch. Int. Med.* 1928: 41: 883. — 38. Maranon, G., Morros, J.: *Endocrinology* 1929: 13: 564. — 39. Coggeshall, C., Root H. F.: *Endocrinology* 1940: 26: 1. — 40. Atkinson cit. by Grafe, E., Tropp, C.: *Handbuch d. Inn. Med.* 3. Aufl. 1944: 611, 513. Ed. by Mohr, L., Staehelin, R., v. Bergmann, G.. — 41. Grafe, E., Tropp, C.: *Handbuch d. Inn.*

- Med. 3. Aufl. 1944: 6 II: 513. — 42. Cushing, H.: Journ. Am. Med. Ass. 1932: 98: 2022. — 43. Bauer, J.: Klin. Wochenschr. 1935: 14: 364. — 44. Simmonds, M.: Deutsch. Med. Wochenschr. 1914: 40, I: 322. — 45. Fröhlich, A. cit. by Hegler, C.: Neue Deutsche Klinik. Berlin & Wien 1930: 5: 237, 238. — 46. Dale, T.: Nord. Medisin 1942: 13: 429. — 47. Leyton, O., Simpson, S. L.: Brit. Encyclop. of Med. Practice. London 1936: 1: 238. — 48. Umber, F.: Die Stoffwechselkrankheiten. München 1925: 59. — 49. Ralli, E. P.: Arch. Int. Med. 1931: 47: 329. — 50. John, H. J.: Arch. Int. Med. 1926: 37: 489. — 51. Gripwall, E.: Acta Med. Scand. 1937: 92: 195. — 52. Zondek, H., Kaatz, A.: La Presse Médicale 1938: 2: 1835. — 53. Oppenheimer, A.: Klin. Wochenschrift 1930: 1: 17. — 54. Salmon, A.: La Presse Médicale 1939: 1: 1033. — 55. Himsworth cit. by Duncan, C. G.: Diseases of Metabolism. Philadelphia & London 1942: 715. — 56. Mainzer, F.: Schweiz. Med. Wochenschrift 1936: 17: 546. — 57. Kylin, E.: Nord. Med. Tidsskrift 1935: 9: 530. — 58. Cushing, H.: Brit. Med. Journ. 1927: 2: 1, 48. — 59. Cushing, H., Davidoff, L. M.: Arch. Int. Med. 1927: 39: 673. — 60. Chabanier, H., Puech, P., Lobo-Onell, C., Lelu, E.: La Presse Médicale 1936: 986. — 61. Hutton, J. H.: American Journ. of Roentgenology 1936: 35: 813. — 62. Lyall, A., Innes, J. A.: The Lancet 1935: 1: 318. — 63. Kotte, J. H., Vonderahe, A. R.: Journ. Am. Med. Ass. 1940: 114: 950. — 64. Rosendahl, G.: Acta Med. Scand. 1927: 66: 100. — 65. Joslin, E. P.: The Treatment of Diabetes Mellitus. Phil. & New York 1917: 68, 338, 409. — 66. Motzfelt, K.: Acta Med. Scand. 1932: 77: 463. — 67. John, E. P.: Endocrinology 1925: 9: 397. — 68. Allan, F. N., Rowntree, L. G.: Endocrinology 1931: 15: 97. — 69. Feldman, F., Roberts, J. B., Susselmann, S., Lipetz, B.: Arch. Int. Med. 1947: 79: 322. — 70. Aron, M.: Compt. rendus de la Soc. de Biol. 1929: 102: 682. — 71. Loeb, L., Bassett, R. B.: Proc. Soc. f. Exp. Biol. and Med. 1928—29: 26: 860. — 72. Means, J. H.: The Thyroid and its diseases. Phil. Montreal & London 1937: 201. — 73. Houssay, B. A.: Endocrinology 1934: 18: 409. — 74. Kemp, T., Okkels, H.: Lærebog i Endokrinologi. København 1937: 38. — 75. Joslin, E. P., Lahey, F. H.: Am. Journ. Med. Sciences 1928: 176: 1. — 76. Frisk, Rune A.: Svenska Läkartidn. 1944: 20: 1396. — 77. Nielsen, H.: Klinisk Endokronologi. København 1938: 1: 250. — 78. Leth Pedersen, A.: Nordisk Medisin 1946: 29: 1571. — 79. Jensen, A.: Ugeskrift f. Læger 1945: 107: 528. — 80. Snapper, I., Groen, J., Hunter, D., Witts, L. J.: Quarterly Journ. of Medicine. New series 1937: 6: 195. — 81. Meyer, F. A., Melzl, E.: Berichte über d. gesamte Physiol. 1942: 129: 619. — 82. Broesameln: Deutsch. Arch. Klin. Med. 1921: 137: 299. — 83. Heni, F.: Klin. Wochenschrift 1939: 2: 1052.
-

From the Medical Service of the County Hospital, Maribo, Denmark.
(Chief: Erling Lundsteen, M. D., Sc. B.)

A Case of Perniciosiform Anemia in a Child Nineteen Months Old.

By

P. H. D. WAAGSTEIN.¹

(Submitted for publication November 10, 1947.)

The existence of pernicious anemia in childhood is contested by many authorities (Bachman, Bass), while others (Karlström & Nordenson, Murray, Petersen & Dunn) maintain that such cases may occur. The whole matter is in a way a question of how one defines the term »pernicious anemia», and especially of whether one requires that the criterion of an achylia resistant to histamine be satisfied. H. K. Faber, in 1928, described a case, in a child nine and a half years old, which is probably the first one found in the literature which fulfilled all the criteria for pernicious anemia. But whether these hyperchromic, macrocytic anemias be considered as true pernicious anemias or not, they respond excellently to liver therapy (Faber, Gerbasi, Langmead & Doniach, and others). According to Petersen & Dunn, the use of the term »pernicious anemia» should be restricted to those cases which fulfill the following criteria:

1. Macrocytic anemia.
2. Gastric achlorhydria resistant to histamine stimulation.
3. Arrest of maturation in the bone marrow at the megaloblastic level.
4. Specific response, *i. e.* reticulosis, following liver therapy.
5. Necessity of continuous therapy to maintain a continuous remission.

I believe that the following report may be of interest, as it represents a borderline case in the standing controversy.

¹ Jakobshavn, Greenland.

Case Report.

The patient was a boy, nineteen months old, born April 4th, 1945, admitted to the service November 18th, 1946 (Record no. 5/47) with the diagnosis severe anemia, acute leukemia. The parents, who were first cousins, were healthy, the mother thirty years old. The patient, who had been born at full term with normal delivery, weighing 3,600 g at birth, was the second of two sons; the elder brother was seven years old and well.

According to the mother's statements and the health nurse's journal, the baby had been only breast-fed until 10 months old, from then until 14 months old chiefly breast-fed, with addition of a portion of gruel daily. After the lactation had ceased, he was put on the usual spoon-fed baby's diet, though with some restrictions. Thus, his mother could not make him eat bread-and-butter with anything laid on, he did not tolerate eggs, because they gave him itching skin eruptions, he did not like minced fish; minced meat was not tried. He was given from one half to three quarters of a liter milk daily, mostly prepared as gruel; also codliver oil; and

Until he was 1 year old, he seems to have developed normally. At 9 months he had four front teeth and could stand on his feet; at 11 months he had all his front teeth and could walk, at 12 months he began to be able to speak, and weighed 9,150 g.

The mother's great problem had all the time been his sluggish stools. She had made extensive use of soap pills and water enemas, and in the last six months before admission she had given him a child's spoonfull of paraffin oil daily. In March, 1946, he had pneumonia, for which he was given lucosil, about 18 tablets (*i. e.* 9 g) in all, whereupon the temperature became normal. At the same time, he began to have alternatively diarrhea and obstipation. The physician called in put him on a grated apples and water formula, but as the child would not eat apples, he practically got no food during those days. The mother then began to give him a diet of weak tea and rusks, and after a while a little gruel. This was repeated every time he had a turn of diarrhea. During all this, the boy became listless and pale, and in May to June a very distressing stomatitis supervened, which resisted all treatment, but at last subsided spontaneously. The fall then passed with alternating periods of obstipation and diarrhea, during which the boy was practically starved, though his grandmother kept insisting on the mistake of not giving him any substantial food. Gradually, he became extremely pale, and the parents got more and more worried. During the last week before admission, his father got the idea that the boy should be given a portion of oatmeal porridge and 20 drops of *Guttae Ido B* daily. The mother stated that he had become so weak and delicate that he caught cold, with cough and coryza, at the slightest exposure.

Physical examination. — The patient weighed 8,510 g, his height was 77 cm. The skin and mucosae were very pale. There were no sure

keratoses or pigmentations. On the right cheek and the left hand there was a slight impetigo. The eyes were natural. There was nothing abnormal in the fauces; the tongue was red; there was no papillary atrophy. All the front teeth and the lower molars were present, the other teeth had not broken through yet. Stethoscopy of the lungs and heart showed normal conditions. The abdomen was soft, there was no enlargement of the organs or any abnormal tumefactions. The right testis was not in the scrotum. The muscles of the extremities were of natural fullness, the reflexes normal.

Laboratory findings. — Gastric secretion fetched up after injection of histamine contained acid. Congo/Phenolphthalein 35/40. The feces looked normal, and at microscopy after sudan staining no fat was found. The reaction to Moro- and Mantoux tests was negative. Also the result of Wassermann test in the mother was negative. Roentgen examination of the lungs showed on the right side a slight accentuation of the hilar tracing, which aroused suspicion of incipient perihilar infiltration. At repeated examination ten months later, the picture was unchanged.

Hematologic findings. — Hemoglobin 30 per cent. Red cells 900,000 per cubic millimeter. Color index 1.45. Volume index 1.61. White cells 7,640 per cubic millimeter. Differential count: staff cells 2 per cent, polymorphonuclear neutrophils 12 per cent, lymphocytes 84 per cent, monocytes 1 per cent, reticulocytes 0.4 per cent. There was marked anisocytosis and poikilocytosis. The diameter of the erythrocytes varied, from 2 to 9—10 microns. There were many macroblasts and macrocytes. Histologic examinations of smears from the bone marrow (Dr. Soeborg Ohlsen) showed:

	November 21st.	December 4th.
	%	%
Myeloblasts	2.0	7.25
Promyelocytes	0.25	2.75
Neutrophile myelocytes	58.25 (5 in mitosis)	12.75 (1 in mitosis)
Eosinophile " 	2.75	2.0
Basophile " 	—	—
Metamyelocytes	+	+
Staff cells	10.75	36.25
Polymorphonuclear neutro- phile leukocytes	5.0	31.25
Eosinophile " 	2.5	1.75
Basophile " 	—	—
Lymphoblasts	—	—
Lymphocytes	18.5	6.5
Monocytes	—	—
Plasma cells	—	—
Megacaryocytes	—	—
Reticulum cells	—	—

Per 400 cells of the white blood system were found:

	November 21st.	December 4th.
	%	%
Erythrogonia	4	8
Erythroblasts, basophile ...	2	23
» , eosinophile ..	18	36
Megalogonia	135	2
Megaloblasts, basophile (1 in mitosis)	55	1
Megaloblasts, eosinophile ...	32	2

The histologically treated coagulum contained marrow particles solely consisting of cell tissue. The most characteristic feature was the hyperplastic, megaloblastic erythropoiesis. Moreover, there was very marked hyperplasia of the myelocytic elements.

Course and Treatment. — As the anemia was so pronounced and the child's general condition so poor, injections of a concentrated liver preparation (*Exhepa fortior*, 2 ml, 4 times in all) were given as soon as the perniciosiform blood picture had been demonstrated. The effect noticeable. His cheeks got red, he became livelier and began to gain in weight. During his stay in the hospital he was given the usual diet for spoon-fed infants, besides codliver oil and ferroplex (1 tablet three times daily). As the condition was diagnosed as deficiency anemia, the liver therapy was suspended, but treatment continued with Bevitall, a yeast autolysate containing all the known components of vitamin B₁ except folic acid. There were no dyspeptic troubles, he ate well, and the stools were normal and spontaneous.

At discharge, November 18th, 1946, he weighed 8,950 g (gain 440 g). Hematologic examination on December 14th showed red cells 4,200,000 per cubic millimeter, color index 1.1. White cells 16,240 per cubic millimeter, with 2 per cent staff cells, 44 per cent polymorphonuclear neutrophils, 8 per cent eosinophils, 42 per cent lymphocytes, 2 per cent plasma cells. No immature cell forms in the peripheral blood. The red blood picture had become almost normal.

When seen for control December 18th, a month after discharge, he was thriving well and weighed 10 kg. Hemoglobin was 92 per cent, red cells 4,340,000 per cubic millimeter, color index, 1.0, volume index 1.0. White cells 12,960 per cubic millimeter, with polymorphonuclear neutrophils 35 per cent, staff cells 2 per cent, eosinophils 3 per cent, lymphocytes 60 per cent. The red blood picture normal.

When next seen for control, May 10th, 1947, the child had lost weight, was now weighing only 9.8 kg. The mother complained that he had lost appetite, and there had again been diarrheas, alternating with periods of obstipation. Moreover, there had again been stomatitis. The mother had followed the hospital's prescriptions for his diet closely, and had given him food rich in vitamin B₁. Hemoglobin 53 per cent, red cells 1,930,000 per cubic millimeter, color index 1.33, volume index 1.4. White cells 5,560 per cubic millimeter. Differential count showed

polymorphonuclear neutrophils 27 per cent, lymphocytes 67 per cent, monocytes 2 per cent, staff cells none. The diameter of the erythrocytes varied from 2 to between 11 and 12 microns. The larger erythrocytes were oval and well filled with hemoglobin. Moreover, there was very pronounced anisochromia, poikilocytosis and a few macroblasts and erythroblasts.

Curve showing Gastric acidity.

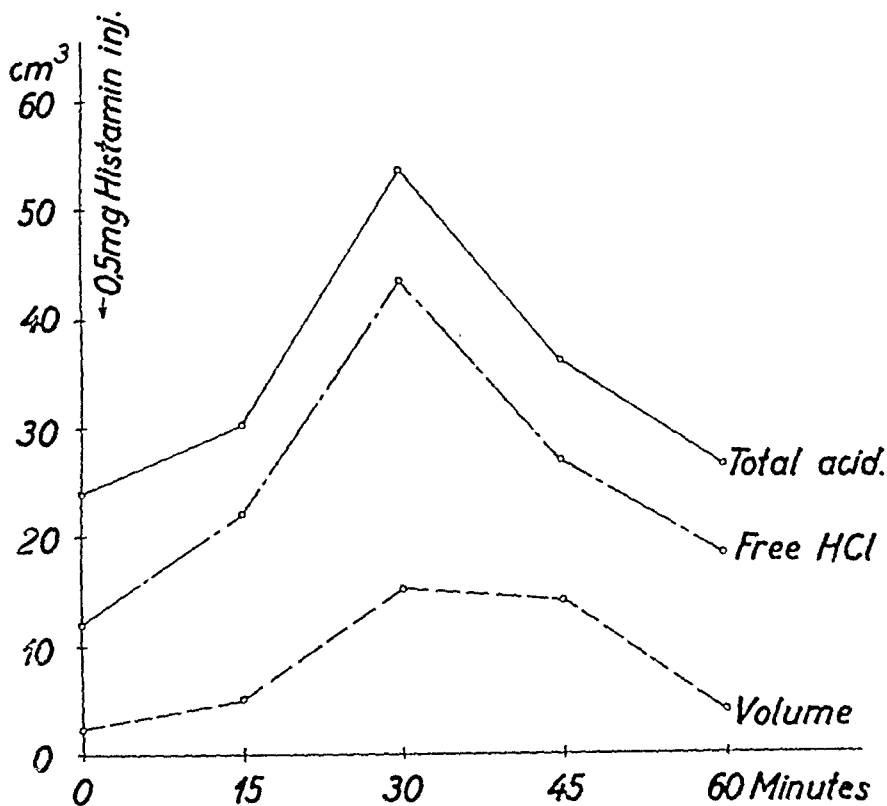


Fig. 1.

On account of this development, the patient was re-admitted. Fractional analyses of gastric secretions showed abundant acid (see Fig. 1). In spite of ferroplex and peroral and parenteral administration of Bevital, there was continued loss of weight, the hemoglobin level dropped, and not until liver therapy was again instituted did remission set in and the child was discharged in a state of progredient improvement, with prescription for continued liver treatment at home.

When seen again for control August 13th, 1947, he had been getting along nicely and had gained in weight. He had been given Exhepa fortior, 2 ml every two weeks. Hemoglobin was 92 per cent, red cells 4,340,000 per cubic millimeter, color index 1.0. White cells 15,640 per

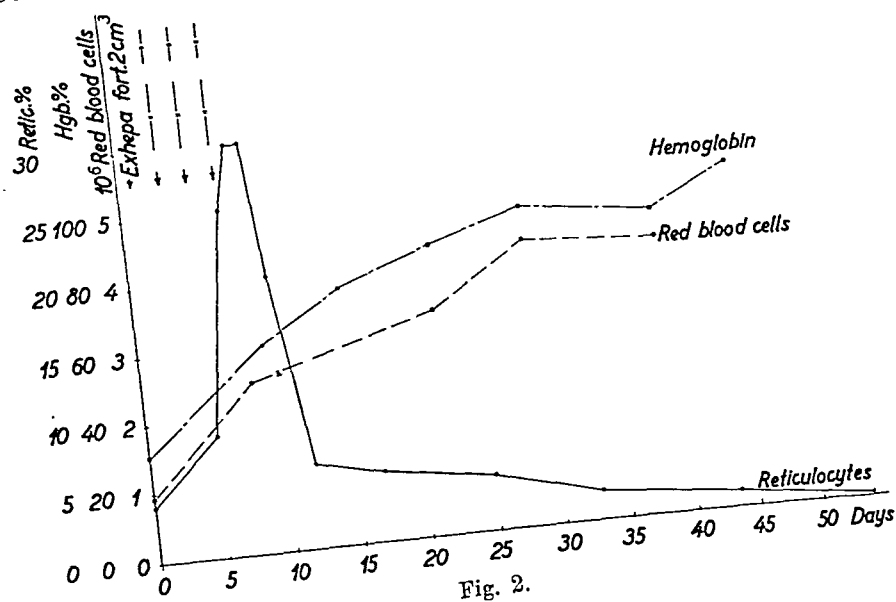


Fig. 2.

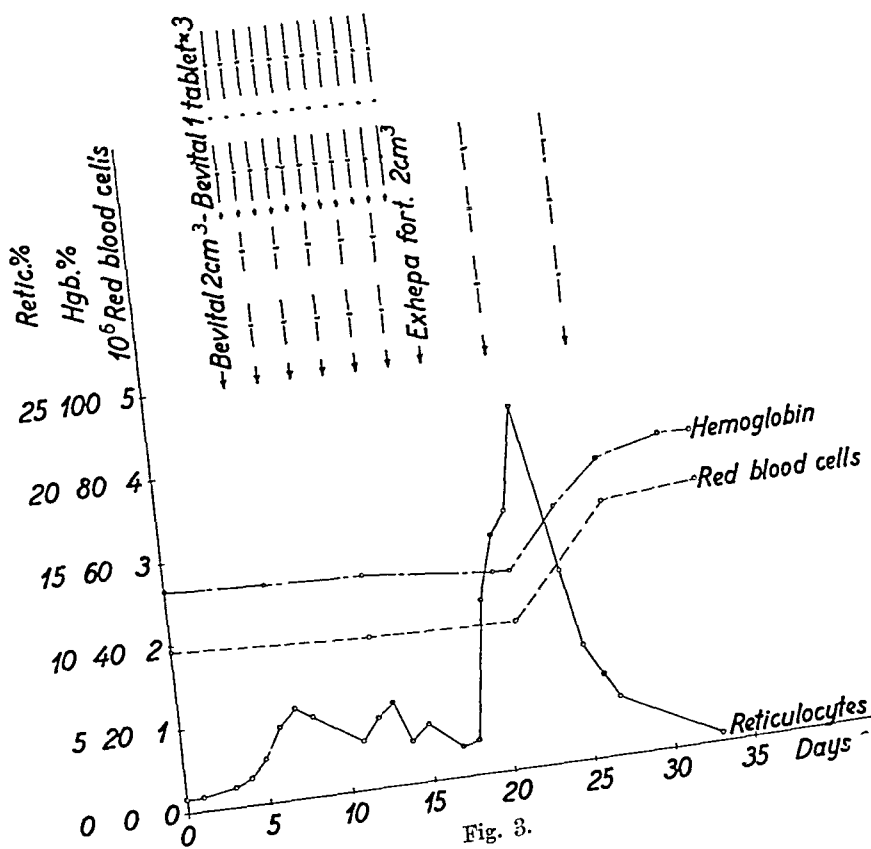


Fig. 3.

cubic millimeter. Differential count showed staff cells 4 per cent, polymorphonuclear neutrophils 38 per cent, eosinophils 2 per cent, lymphocytes 56 per cent. There were no immature cells in the peripheral blood. The red blood picture had become almost normal.

Discussion.

A characteristic feature of the case was the infection, followed by gastrointestinal disturbances, and in this respect it quite resembles the cases reported by Bachman and Bass. According to Bachman's theory, the macrocytic anemia should be due to inadequate nutrition, combined with low acid values owing to the infection. It must also be considered if the preceding sulfonamide treatment may not have been a contributing causative factor. According to American studies, certain bacteria in the human organism are capable of synthesising aneurine and nicotinic acidamine, but the process can be prevented by certain sulfonamides (Najjar & Holt; Najjar; Johns; Fleischman & Holt; cit. by Schönheyder).

The literature contains many reports of macrocytic anemias due to inadequate nutrition. Wills & Evans have reported cases from India, but also in the southern States of the U. S. A. many cases are said to occur (cit. from Hagtvedt). In our latitudes, the occurrence of macrocytic anemia is rare (Alsted; Groen & Snapper). In our case, B-vitamins had no effect on the anemia, no matter whether administered perorally or parenterally. Only liver seems to contain the substance necessary for the normal erythropoiesis. If we hold to the stringent criteria for pernicious anemia specified above, the present case cannot be accepted as pernicious, though liver alone contains this specific substance. The gastrointestinal symptoms, the stomatitis and the macrocytic anemia make one think if this substance might not be the *lactobacillus casei* factor, *alias* folic acid, *alias* vitamin M, *alias* vitamin B₁₂.

Megaloblastic anemia is probably not very rare, either; at least Zuelzer & Ogden have recently reported twenty-five cases from the U. S. A. They consider the type to represent a special form of reaction in young organisms. The cases in which the patient did not succumb to the infection were cured in very short time with folic acid, some even without the employment of any therapy at all. Our case has now been followed for ten months, and so far it does not seem that the child is able to get along without the

liver treatment. Peterson & Dunn followed a case of a child thirteen months old at first admission for five years, and believe that it must be considered as one of true pernicious anemia.

Summary.

The author reports a case of megalocytic anemia with free acid in the stomach, in a child nineteen months old, and discusses the etiology and pathogenesis of anemia. Diet suitable for the age of the patient, together with iron, B-vitamins and Exhepa fortior brought speedy recovery, but cessation of the liver therapy soon resulted in relapse despite an otherwise sufficient diet; and only resumption of the liver treatment brought abatement of the symptoms.

Literature.

Alsted, G.: *Am. J. med. Sci.* 1939: 197: 741. — Bachman, A. L.: *Am. J. Dis. Child.* 1936: 52: 633. — Bass, M. H.: *Am. J. Dis. Child.* 1944: 67: 341. — Faber, H. K.: *Am. J. Dis. Child.* 1928: 36: 1121. — Gerbasi, M.: *Pediatrics* 1929: 37: 1343. — Green, J. & I. Snapper: *Am. J. med. Sci.* 1937: 193: 633. — Hagtvedt, J.: *Nord. Med.* 1946: 31: 1867. — Karlström, F. & N. G. Nordenson: *Acta Paediatrica* 1944: 32: 58. — Langmead, F. S. & J. Doniach: *Lancet* 1937: 5931: 1048. — Murray, U.: *Sv. Läk.* 1947: 7: 411. — Peterson, J. C. & C. Dunn: *Am. J. Dis. Child.* 1946: 71: 252. — Schonheyder, F.: *Nord. Med.* 1946: 32: 2573. — Wills, L. & B. D. F. Evans: *Lancet* 1938: 2: 416. — Zuelzer, W. W. & F. N. Ogden: *Am. J. Dis. Child.* 1946: 71: 211.

From the Department of Physiology, University of Oslo.

Studies of Erythrocyte Counting.¹

II.

Technical-Physiological Errors.

By

HENRIK F. LANGE and HERBERT PALMER.

(Submitted for publication November 21, 1947.)

In a previous publication we have dealt with some *purely technical* sources of error in erythrocyte counting. In this article we will discuss some points in the technique of taking blood samples.

When blood capillaries are severed, there immediately sets in a reaction of the blood vessels. This is manifested by a spasm in the damaged capillaries, so that the circulation therein practically ceases. The blood is conveyed over to neighbouring capillaries through intercapillary anastomoses.

The spastic period is of varying duration, usually from 10 to 30 seconds. Clinically, this period is characterized by scanty bleeding.

During this time the reparatory processes have begun, with softening of the vascular wall at the site of injury, and have supplied the basis for the thrombocyte-thrombus formation that gives rise to the intravasal hemostasis. In the blood that oozes out and stands in contact with the cut-surface, the coagulatory processes come into action.

In normal individuals this physiological hemostasis is complete after 3 or 4 minutes (»the bleeding time«).

¹ The work has been supported by grants from Freia Chocolate Fabriks Medicinske Fond.

In view of these circumstances we can therefore expect to find only a very short period of normal blood-flow outward, *i. e.*, a flow of blood which in quality and quantity corresponds to the intravasal blood current under physiological conditions. In practice, we can therefore speak of normal flow of blood for a short time after the first spastic period is over, as will be seen in one of the later-described experiments.

The most practical mode of procedure for ascertaining the influence of the different reactions on the erythrocyte values will then be to make examinations of the blood samples as frequently as possible after the puncture and to compare these with a «constant blood», for example, venous blood.

Methods.

With sufficient assistance and precaution we were enabled to make examinations at average intervals of from 10 to 15 seconds. The first examination could in general be made 8 seconds after the puncture (we are here reckoning with the time until the blood is drawn into the pipette and transferred to the diluting liquid). In this manner we usually were able to make from 8 to 10 examinations without difficulty, the last examination being made about 150 seconds ($2\frac{1}{2}$ minutes) after the puncture. After that time the reparatory processes at the site of puncture began to come into action (fibrin filaments, small coagula, poor flow of blood). Blood samples were taken from ears and fingers.

As basis of comparison was used venous blood taken from the median cubital vein through puncture with a Wassermann cannula. Tourniquet was not used for these punctures.

The venous blood values remain constant during the period under considerations, as is shown by the following experiment: In three persons venipuncture was made every second minute during 10 minutes (6 punctures in each person). The average erythrocyte values are given in Table 1.

Table 1.

Result of Erythrocyte Counts in Serial Examinations of Venous Blood.

Time in min.	0	2	4	6	8	10
Erythrocytes	4.643	4.638	4.628	4.633	4.646	4.639

In the following diagrams we have therefore found it permissible to employ a straight line to denote the venous blood value. It is to be noted that the individual erythrocyte values in venous blood showed no greater variations than are recorded in the table of averages.

When using the above-described method we found it of importance to consider the following problems (named in «chronological» order).

1. Influence of skin-cleansing by ether («hyperemisation» or not?).
2. Choice of puncturing instrument.
3. Influence of pressure around the site of puncture (for expression of blood).
4. Effect of wiping off the first drop of blood (as is often done).
5. Choice of ear-blood or finger-blood.
6. At what period of time after the puncturing can the capillary blood be regarded as «normal blood»?

Of these questions the last two were found to be of greatest importance, and therefore the chief attention was devoted to them. The first four questions were made the subject of orientative investigations.

Cleansing of the Skin.

Ether is usually employed for disinfection of the part of the skin in which the puncture is to be made. In addition to disinfection, it is generally sought to produce a so-called «hyperemia» at the site of the puncture by massaging with the ether-soaked wad of cotton, especially if skin is pale. It is sought to attain a red and «hyperemic» finger-tip or ear-lap.

In order to ascertain whether this «hyperemisation» has any influence on the result of the count, three persons were investigated for that purpose.

Technique: One finger-tip was massaged for about 15 seconds with a wad of cotton soaked in ether, while another finger-tip was merely washed over («touched») with the cotton-wad (in the first four experiments only finger-blood was used).

The results are shown in Diagram 1.

As is seen, all the erythrocyte values are somewhat lower where the finger has been rendered hyperemic. It is also seen that this

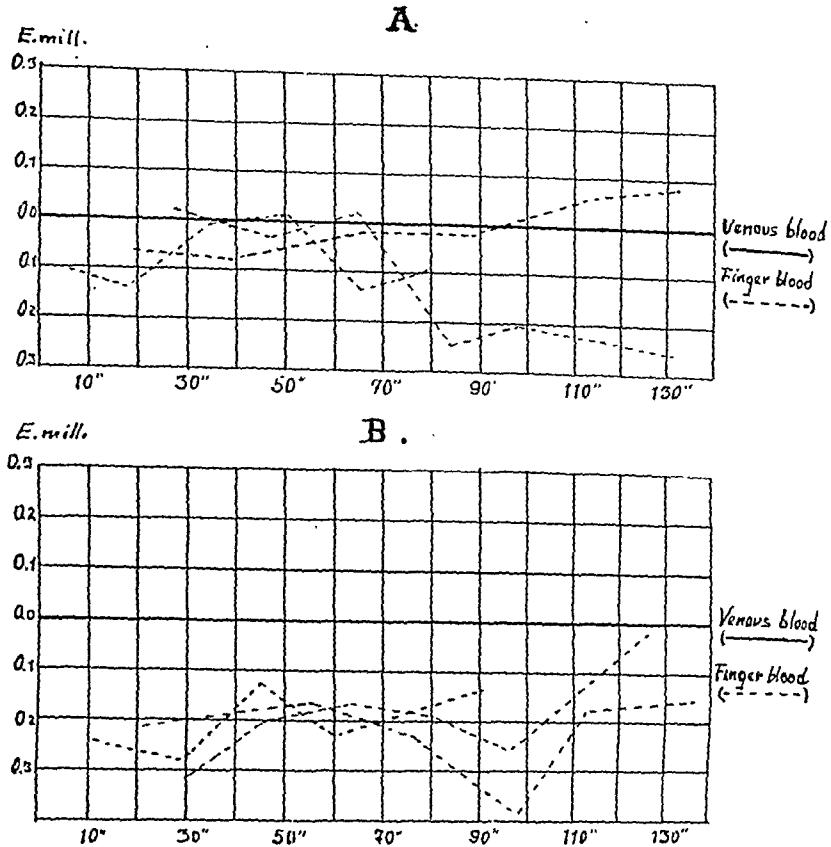


Diagram 1.

The results of frequent erythrocyte counts in finger-blood after washing slightly with ether (A) and after vigorous hyperemisation (B).

effect persists for nearly 2 minutes (about 110 seconds) after the puncturing.

It is then found that the finger-blood after hyperemisation shows on the average a 5 per cent lower content of erythrocytes than finger-blood without hyperemisation.

This may be explained by the assumption that on hyperemisation there occurs a temporary dilatation of the capillaries through oozing in of fluid from the tissues, whereby the blood is diluted.

The fall in erythrocyte content is not great, but it represents a source of error that may easily be avoided. It is to be supposed that the same effect will be produced on use of other methods for rapid hyperemisation of the skin. Meanwhile, it indicates that skin ought to have a »standard» temperature, if correct results are to be attained.

Although the investigations are few in number, yet they go to show that massage of the site of puncture ought to be avoided.

Instrument Used for Puncturing.

Our impression was that the erythrocyte values were a good deal less uniform when a scarificator was used for puncturing than when we used a sharp knife.

With use of the same technique as before, it was found that in three subjects of experiment the *average deviation* from the venous blood value (taken as 0-point) was 58 per cent greater after puncturing with scarificator than after puncturing with knife, and that the *average range of variation* around the true venous blood value was 3 per cent with use of scarificator and 1.9 per cent with use of knife.

This may possibly be ascribed to the construction of the scarificator, which usually have a conical edge. Moreover, the place of puncture is subject to a sudden pressure when the spring is released. For both of these reasons the capillaries sustain more injury than would be caused by a thin narrow knife guided by a light hand.

In our opinion therefore a scarificator is here not very suitable for use in taking blood-samples.

Pressure to Force Out the Blood.

Occasionally the flow of blood from the puncture may be scanty, especially if the incision is small and shallow. It is usual to provoke a better outflow by exerting pressure on the parts around the puncture.

From 5 parallel counts made in order to ascertain the influence of such pressure on the erythrocyte content it was found that the number of erythrocytes rose by from 4.1 to 9.5 per cent, the average rise being 7.5 per cent. In one case, where very strong pressure was employed, there was a rise of no less than 26 per cent.

Accordingly it seems that by pressing out the blood from the puncture there is introduced a source of error that may be very considerable.

The experiments thus confirm the old rule that copious bleeding is necessary.

This is attained by making the puncture a little deeper and broader than is usually prescribed in directions for laboratorial technique.

According to our experience, satisfactory bleeding can be obtained by making the incision about 4 mm deep and 3 or 4 mm wide.

Wiping Off of Blood.

It is usual to wipe off the first drop of blood that appears. This procedure is, quite correctly, defended on the grounds that the first blood that comes has not the right composition.

The question now is whether the wiping away of this first drop of blood leads to new reactions at the site of puncture.

This matter has been investigated in four subjects, with the previously described technique.

The results are shown in Diagram 2 and 3.

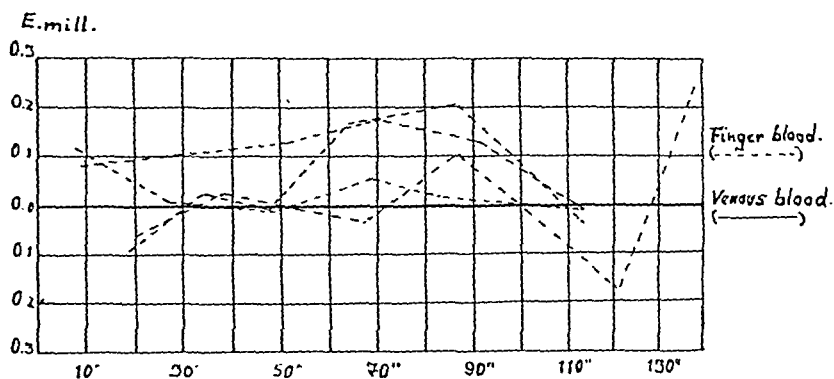


Diagram 2.

Results of erythrocyte-counts in finger-blood, where the first drop of blood was *not* wiped away.

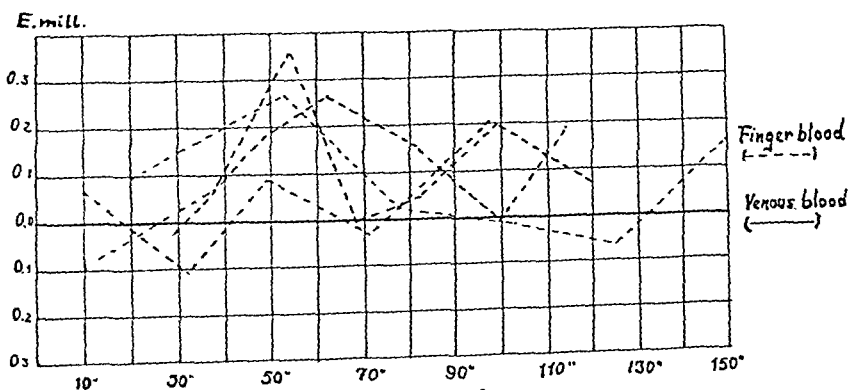


Diagram 3.

Results of similar experiment after wiping off the first drop of blood.

It is seen that the erythrocyte values are less uniform after wiping than when the blood is allowed to flow freely.

Numerically, it is found that the *mean deviation* from the real venous blood value is *2.5 per cent for finger-blood after wiping and 1.8 per cent without wiping.*

Thus there is no justification for wiping off the first drop of blood. On the other hand, it shall not be used for analysis, but shall be allowed to flow away.

The conclusion to be drawn from this experiment therefore is that the blood shall be allowed to flow freely and undisturbed.

Ear-Blood or Finger-Blood?

On this question there has been a lively discussion, which has gone more and more in favour of finger-blood.

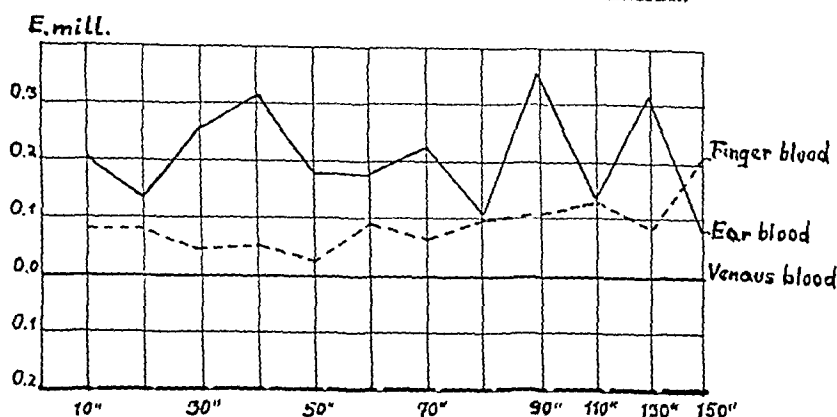
Meanwhile, it is a fact that ear-blood is still being employed in most of the routine investigations. Technically and psychologically, the ear-lobe is a far more advantageous place for the incision than the finger-tip. The patient need not see the knife or the actual puncture, and the ear-lobe is less sensitive than the tip of the finger. It is therefore natural that these points should weigh heavily in dealing with patients.

The question then is, whether ear-blood and finger-blood can be put on a level with venous blood. Venous blood is to be preferred. But the use thereof is limited in practice for several reasons: More circumstantial procedure (including preparation of the syringe etc.), discomfort for the patient from the puncture, difficulty of execution in case of young children and persons with small and deep-lying veins.

The problem: ear-blood or finger-blood? has therefore been investigated with the above-described technique in 10 persons.

The results are given in Diagram 4 and in Table 2.

It is here very distinctly seen that in its content of erythrocytes finger-blood comes very close up to venous blood. Further, that finger-blood is far more stabile in character than ear-blood. (Cfr. Gregers Sørensen: Nord. Med. 1941: 10: 117.) The range of deviation for the ear-blood values stretches from 1.5 to 7.6 per cent, for the finger-blood values from 0.5 to 4.8 per cent.



Average curves (10 observations) of the erythrocyte content in ear-blood (—) and in finger-blood (---) in the first 2 1/2 minutes after puncturing.

Table 2.

Average Percentual Deviation of Erythrocyte Content in Ear-Blood and Finger-Blood Respectively from the Venous Blood Value, Noted at Different Times (Given in Seconds) after the Puncturing.

Time	0— 10	10— 20	20— 30	30— 40	40— 50	50— 60	60— 70	70— 80	80— 90	90— 110	110— 130	130— 150
Diff. venous/ ear-blood....	4.3	2.9	5.7	6.8	4.0	3.9	5.0	2.4	7.6	3.1	6.8	1.5
Diff. venous/ finger-blood .	1.9	4.0	1.1	1.1	0.5	2.0	1.3	2.1	2.4	2.9	1.8	4.8

Period of Time at which the Blood becomes »Normal«.

Meanwhile, it is important to note *when the above-mentioned extreme values appear*, and it is then natural to try to establish in what time after the incision the capillary blood can be regarded as »normal blood«.

We note that the erythrocyte values for ear-blood, besides showing a greater range of deviation from the venous blood values, also display large and irregular (unsystematic) variations during the whole period of observation.

The finger-blood values, on the other hand, show a far more regular variation, with the greatest deviation at the beginning (in the first 20 seconds) and at the end (in the last 20 seconds) of the observation time. This accords well with the clinical ob-

servation: scanty, uneven bleeding in the beginning and restricted bleeding at the end.

On closer examination we further find that in finger-blood the erythrocyte values differ very little from the venous blood value in the period from 20 to 50 seconds after the puncture and that during that time, about half a minute, they remain fairly constant, without any variation. After this period there is seen a greater difference, with a relatively steady tendency to rise.

It seems therefore, in the first place, justifiable to employ finger-blood instead of ear-blood. In scientific work ear-blood must be deemed unsuitable for the counting of erythrocytes.

Secondly, it seems justifiable to regard the capillary blood as «normal» in the period from 20 to 50 seconds after the incision.

As a practical rule it may therefore be said that we should wait half a minute before drawing up the blood into the pipette for examination.

Summary.

An account is given of some investigations respecting the technique adopted in taking blood-samples for the counting of erythrocytes.

After mention of the processes that come into play on severance of capillaries there is described a method of studying, with frequent observations, the influence of these processes on the content of erythrocytes. By use of this method the following results were attained:

1. There is found some reduction (about 5 per cent) of the erythrocytes on hyperemisation of the skin with ether. By simply washing the site of the incision with ether this source of error is avoided.

2. Use of a scarificator leads to greater reflex reactions in the vessels than use of a narrow, sharp knife.

3. Pressure around the site of puncture for the purpose of obtaining more copious bleeding may have a marked influence on the results of the counting. Such pressure is therefore inadvisable, and an incision 4 mm deep and 3 or 4 mm wide is recommended in order to produce satisfactory bleeding.

4. There is found no justification for wiping away the first drop of blood that appears after the puncture. Such a procedure leads to undesirable variations in the number of erythrocytes.

5. It is found that the erythrocyte values in ear-blood are irregular and show a large range of deviation. Finger-blood comes close up to the venous blood value and is free from the great variations shown by ear-blood.

6. It is shown that finger-blood can be regarded as »normal blood» from 20 to 50 seconds after the incision. It is therefore recommended that the blood should be drawn up into the pipette half a minute after the puncture, provided there is free and undisturbed flow of blood during that time.

From the Medical Clinic of the Caroline Hospital, Stockholm.
(Head: Professor N. Svartz.)

Comparative Studies of the Effect of Some Vasodilators in Angina Pectoris.

By

EBBE NYMAN, M. D.

(Submitted for publication December 15, 1947.)

Quantitative comparative studies on the effect of symptomatic medicines in man have hitherto been conducted on far too limited a scale, and this is especially evident when we compare them with the extensive work that has been laid down on animal experiments from the same point of view. A good illustration of this is the work done in connection with vasodilative drugs, the use of which is enormous in practice and is based on traditions dating back ninety years. Thus, amylnitrite was first introduced in 1860, by Lauder Brunton, and quite independently of this, nitroglycerine was also brought out in the same year, by Richardson.

Our defective knowledge regarding the quantitative effect of the different vasodilative drugs in man under physiologic and pathologic conditions is partly to be explained by the lack of objective, reproducible function tests that has hitherto existed.

The vasodilative drugs, of which the nitrite group in its widest sense is of chief interest in this connection, act, as we know, on the finer blood vessels by causing direct relaxation of their muscle tissue. When coronary sclerosis with more or less rigid vessel segments is present, the effect must be attributed to dilatation of non-rigid collateral vessels, which are often present in large numbers. The fact should, furthermore, be stressed that the value

of nitrites in angina pectoris is not in itself connected with the depressor action which is one of the most striking pharmacodynamic effects of these substances.

The value of nitrites in angina pectoris thus does not originate directly from their depressor action in hypertension, in essential hypertension, for instance. The investigations on man on which the current opinion as to the effectiveness and duration of action of these substances is based have been carried out mainly on series of patients with hypertension. On the basis of these findings, nitrite has been grouped in a series in which the quickest and shortest effect is achieved with amyl nitrite and where the isomannide and sorbide compounds are those taking the longest time to take effect and lasting the longest. Nitroglycerine lies fairly close to amyl nitrite, while sodium nitrate, erythrol tetranitrate, pentaerythrite tetranitrate, and mannitol hexanitrate occupy an intermediate position in both these respects. For details, the reader is referred to Goodman and Gilman (1941), Flodmark and Wramner (1943), Goldberg and Porjé (1946), and others.

The sparsely reported investigations¹ on the effect of nitrite compounds on the working capacity of patients with angina pectoris, where the function problem, in other words, has been the main subject of study, are of particular interest in the present connection. Thus, Wayne and Laplace (1933) found that nitroglycerine, in a dose of 0.65—2.0 mg, increased the work tolerance by between 30 and 150 per cent in 10 out of 11 cases of angina pectoris, and that in every case the pain provoked by the work was of shorter duration than it would otherwise have been. The function test used in this series was walking on a staircase. Master, Jaffe and Dack (1939), on the other hand, found that in a little over 100 polyclinic patients with angina pectoris nitroglycerine and sodium nitrite, among other compounds, gave no better results than placebos. The calibrated and reproducible tests for cardiopulmonal function that are now available allow us to study this question more satisfactorily.

The Author's Investigation.

The cardiopulmonal function test employed in the investigations to be described here is the test that has been in use for

¹ After this paper was written, an investigation concerning the effect of theophylline and nicotine acid was published by Suopanki in *Nord. Med.* 1947, 36, 2317.

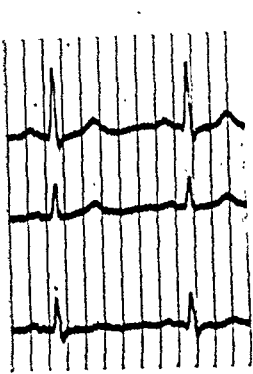


Fig. 1.

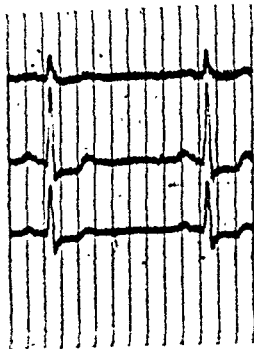


Fig. 2.

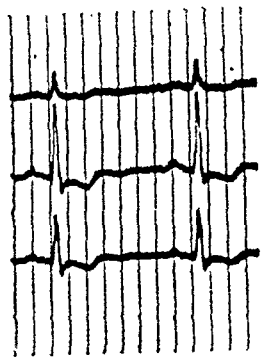


Fig. 3.

Fig. 1. ECG at rest.

Fig. 2. ECG immediately after work, 600 kgm/min for 4 min.

Fig. 3. ECG 3 min. after work.

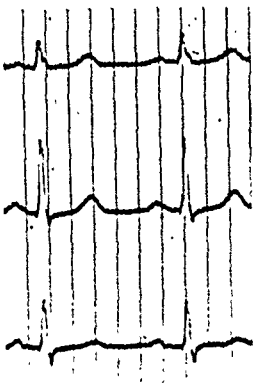


Fig. 4.

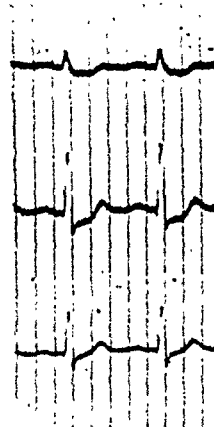


Fig. 5.

Fig. 4. ECG immediately after hypoxemia test. (9% O₂ in N₂ for 8 min.).

Fig. 5. ECG immediately after hypoxemia test and chilling (9% O₂ in N₂ during 8 min. and a piece of ice in one hand).

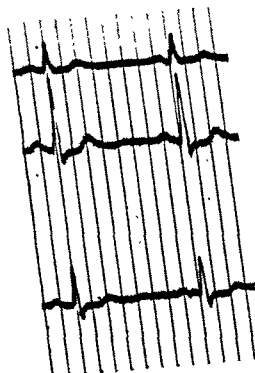
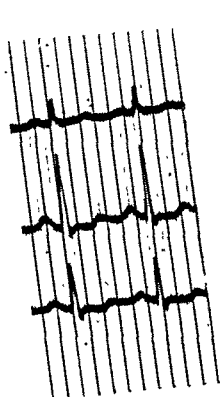


Fig. 6.

Fig. 7.

(For figs. 6—19, see table 1.)

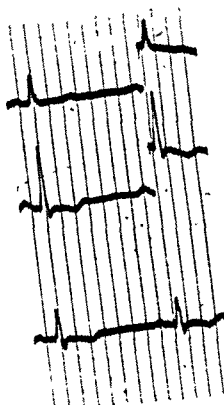
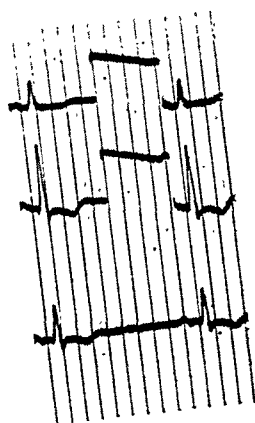


Fig. 8.

Fig. 9.

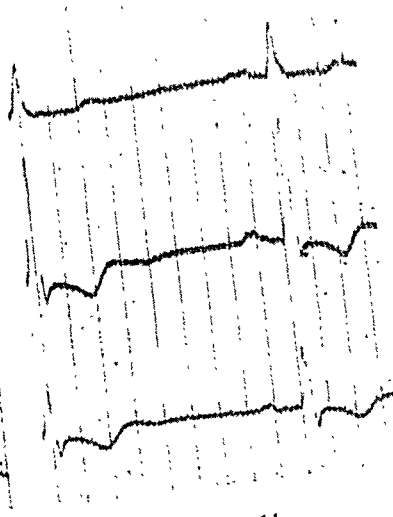
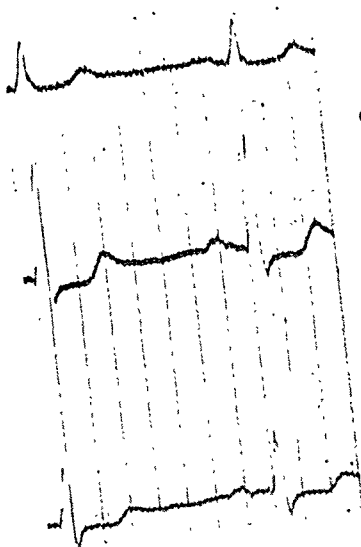


Fig. 10.

Fig. 11.

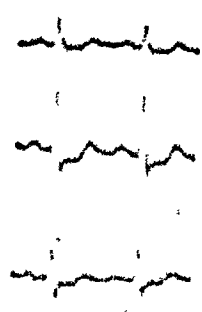


Fig. 12.

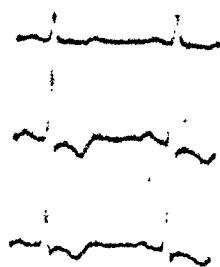


Fig. 13.

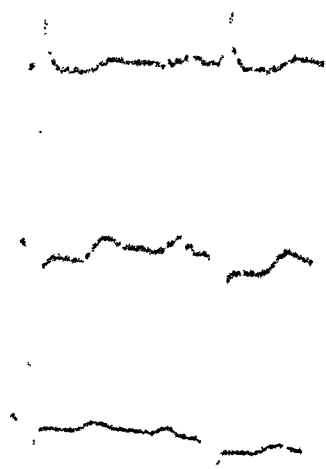


Fig. 14.

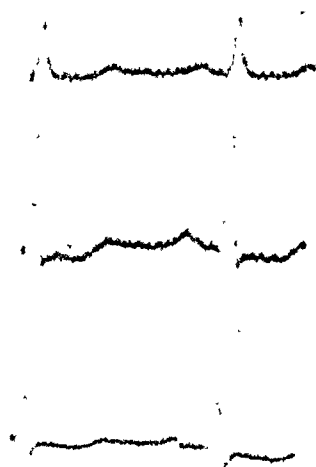


Fig. 15.

Changes in the ECG in Myocardial Infarction.

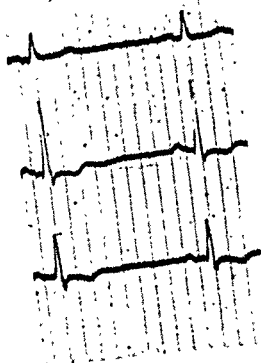


Fig. 16.

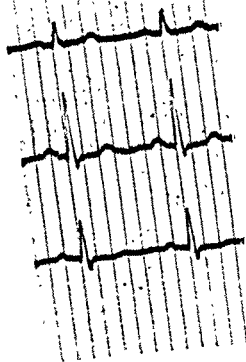


Fig. 17.

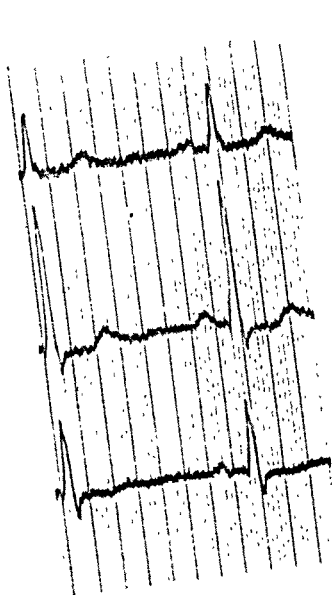


Fig. 18.

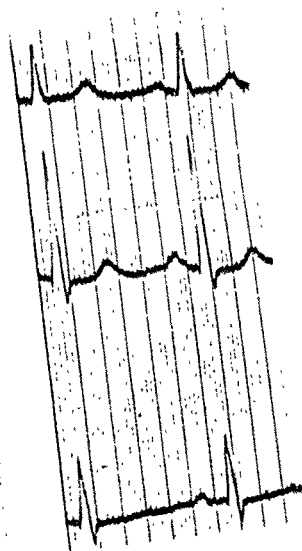


Fig. 19.

Table 1.
Experimental Results.

Drug	Dose & method of administration	Relation of dose to the function test	Work on bicycle ergometer	Appearance of pain in control test before medication. After about	Appearance of pain under influence of drug. After about	ECG immediately after end of work. Fig. no.	ECG about 3 min. after end of work. Fig. no.
Nitroglycerine	0.5 mg by mouth	10 min. before	600 kg /min.	2 min.	4 min.	6	7
Pent-erythrite tetra-nitrate	0.27 g by mouth	1 tabl. of 0.03 g 3 days running incl. test day	600 kg /min.	2 min.	2 min.	8	9
Sorbide dinitrate	0.09 g by mouth	1 tabl. of 0.01 g 3 days running incl. test day	600 kg /min.	2 min.	3 min.	10	11
Theo-phylline ethylene-diamine	1.8 g by mouth	2 tabl. of 0.20 g 3 days running incl. test day	600 kg /min.	1½ min.	3 min.	12	13
Theo-phylline ethylene-diamine	0.24 g intra-venously	15 min. before	600 kg /min.	2 min.	4 min.	14	15
Papave-rinehydro-chloride	0.03 g intra-venously	15 min. before	600 kg /min.	2 min.	2 min.	16	17
Novo-caine	0.30 g by local anesthesia subcuta-neously over the sternum	15 min. before	600 kg /min.	2 min.	3½ min. (pain shifted upward)	18	19

several years at the physiologic laboratory of the Caroline Hospital for the routine clinical examination of certain types of heart disease. This test, which was worked out in principle by Sjöstrand, has been further extended by Wahlund (1945), and consists in dosed work in a bicycle ergometer. In this study, the moderate work of 600 kgm/min. was used. An electrocardiogram was made after 4 minutes' work and again 3 minutes after the work was concluded. The various vasodilators were given according to the

scheme shown in table 1. The whole series of experiments was carried out within the course of one month and the tests were made at the same time each day.

Experimental subject. A 35 year old butler who had had mild polyarthrititis without after-effects at the age of 20, was used as the test subject. He was a moderate smoker. No venereal infection. Since 1943 he had had angina pectoris pains with the typical localization and radiation in connection with rapid walking, running and bicycling uphill. The pains increased in severity in cold and windy weather. Examination showed a heart of normal size and configuration. No physical or roentgenographic signs of organic heart disease. No signs of sclerosis or thrombo-angiitis obliterans in the peripheral vessels. Wassermann's reaction in serum negative. ECG at rest, normal (fig. 1). During the function test (in a bicycle ergometer, 600 kg/min. for 4 minutes) anginal pains developed regularly after about 2 minutes and an ECG showed sure signs of coronary insufficiency (figs. 2 and 3). The diagnosis of disease of the coronary arteries received added support from the hypoxemia test, which showed an unequivocally pathologic picture when it was made more difficult by chilling the patient (figs. 4 and 5).

All the tests with vasodilators were carried out under uniform standard conditions. Control tests were made before each test with the drug; and at each function test performed without previous preparatory treatment with a drug, anginal pain appeared with great regularity after about 2 minutes' work. The results will be best seen from the accompanying table.

Discussion.

The best effect, both on the duration of the pain-free working period and on the ECG, was obtained on the administration of a drug with a rapid action shortly before the start of the function test. Thus, nitroglycerine doubled the length of the pain-free period and had a noticeably favourable effect on the electrocardiogram. Theophylline with ethylenediamine, administered intravenously, also prolonged the pain-free period considerably and the ECG was likewise influenced favourably. Theophylline with ethylenediamine given by the oral route also had a good effect on the pain-free period while the corresponding effect on the ECG was not so striking. Sorbide dinitrate by mouth had some pro-

longing effect on the pain-free period; it did not, however, cause any change in the ECG. Papaverinehydrochloride, administered intravenously, improved the ECG considerably but did not alter the duration of the pain-free period. Finally, local anesthesia with novocaine over the sternum prolonged the pain-free period, shifted the pain higher up, and had some effect on the ECG, which was surprising.

The results do not, of course, allow any far-reaching conclusions to be made. They nevertheless confirm certain empirical experiences with regard to the effect of vasodilators in angina pectoris. One fact that comes out clearly is that a sure effect is only achieved if the drug being tried is given in a readily absorbable form in immediate connection with the function test. With the possible exception of sorbide dinitrate, which seems to possess a fairly high degree of effectiveness, relatively small peroral doses of the drugs with a slower action do not seem appreciably to increase the functional capacity. The dose of theophylline with ethylenediamine per os that was used in these experiments does not justify the drawing of optimistic conclusions, since 0.20 g 3 times a day cannot be used in all patients, owing to toxic reactions. The good effect of nitroglycerine is still further illustrated by these experiments, and likewise that of theophylline with ethylenediamine when administered intravenously.

Summary.

A comparative study of the effect of a number of vasodilative compounds on a young individual with angina pectoris whose coronary function was tested by bicycle ergometry and electrocardiography after the administration of various drugs. Nitroglycerine successfully defended its already well-grounded reputation as a good vasodilator. The effect of compounds having a slower action was not so convincing. Theophylline with ethylenediamine, when administered by intravenous injection, also had remarkably good effect.

References.

- Brunton, T. L.: Lectures on the action of medicines. New York 1897.
 — Flodmark, S. and Wramner, T.: Sv. Läkartidn. 1943, 52. — Gold-
 berg, L. and Porjé, I. G.: Nord. Med. 1946. 29, 190. — Goodman, L.

and Gilman, A.: The pharmacological Basis of Therapeutics. New York 1943. — Master, A. M., Jaffe, H. L. and Dack, S.: Am. J. Med. Sci. 1939, 197, 774. — Wahlund, H.: Nord. Med. 1945, 25, 219. — Wayne, E. J. and Laplace, L. B.: Clin. Sci. 1933. 1, 103.

Chrysotherapy and its "Toxic" Reactions in Rheumatoid Arthritis.

By

GUNNAR EDSTRÖM.

Lund, Sweden.

(Submitted for publication November 28, 1947.)

The three great chronic infectious diseases, tuberculosis, syphilis and rheumatism, have certain clinical features in common. This is so because many dominating clinical manifestations of these diseases are nothing but secondary manifestations in the morbid process, they are allergic or hyperergic reactions. The common features and syndromes are the pronounced hyperergic ones. The differences between the clinical pictures, on the other hand, are due to the etiological factors. More and more evidence has been brought to light indicating that streptococci, especially β -haemolytic, play an important rôle in the etiology of rheumatic infectious processes, both the rheumatic fever and the rheumatoid arthritis (sciatica, arthrosis, and so on do not belong to true rheumatic infection).

In the case of rheumatic infectious process — as well as in the other chronic infections — the clinical picture, however, is characterized not so much by the etiological factor as by the secondary reaction, the allergic reaction, sometimes extended to metal-
lergic or pathergic reaction, and this allergy is not equally pronounced in all the clinical pictures. In the clinic we come across the whole scale from the most turbulent hyperergy to true anergy. The highest notes, representing the most turbulent hyperergy,

fall within the area of rheumatic fever, but the long scale of diminishing allergic reaction down to true anergy is present in the extensive group of rheumatoid arthritis, thereby creating variable clinical pictures of disease (Edström, 1940).

Therapy must therefore be directed partly against the origin, the etiology, of the rheumatic infection, partly to bring about a blunting of the hyperergy and an alleviating of the patient's most troublesome symptoms, which mostly stand in close connection with this hyperergy.

The effect of salicylates and amidopyrine on these conditions of disease lies in the fact that they blunt the hyperergy, they close the capillary walls and suppress the tissue oedema (Eppinger, 1935, Fischer & Wersig, 1936, Dworacek, 1939). They therefore have a good effect on turbulent cases of rheumatic fever but in most cases not so good an effect on rheumatoid arthritis.

Much of our symptomatic therapy in these conditions, such as treatment with hot baths and mud-packs, is also directed principally against one of the most striking hyperergic features in the clinical picture, the peripherally impaired circulation (Edström, 1940).

In our anti-infectious therapy at present the treatment with gold salts plays the most important rôle. Treatment with sera and vaccines has not given so good results, and nor has the administration of sulfonamides, penicillin, streptomycin. The gold salts produce their best results in rheumatoid arthritis but have little effect on the turbulent symptoms at onset of rheumatic fever.

Gold salts however, do not form the ideal anti-infectious drug we are looking for in rheumatoid arthritis because

1. they are toxic;
2. not all patients respond to them;
3. in a moderate percentage the arthritis recurs following cessation of chrysotherapy (Comroe, 1944).

The »toxic» reactions are the biggest crux. They come quite irregularly, may occur following even small doses, and their severity and duration are very capricious. According to our experiences, they seem to be not so much toxic as a result of hypersensitiveness of the organism towards the drug.

To find out whether there may be some connections between the gold concentration in the organism and the excretion of the gold during the gold therapy and these »toxic» reactions we have, at

the Rheumatological Department of the University Hospital in Lund, investigated the concentration of this substance in plasma and urine during gold therapy in cases of rheumatoid arthritis with and without such «toxic» reactions.

To determine the gold concentration we have used a spectrophotometric method on the same principles as Gantzel & Larsen (1943). We determined the gold level in the plasma and urine. Most of the excretion of gold from the organism passes through the kidneys (Freyberg et al. 1941). The gold preparations used have been Solganal B oleosum (aurothioglucose) or Neosolganal (aurokeratinate).

Thirty-three cases have been followed in this way with determinations of the gold level in the plasma and urine during gold-therapy. Eight of them had «toxic» reactions, six of these dermatitis, one had gastro-intestinal symptoms and one transient albuminuria. Two of the dermatitis-cases were severe with bad pruritus and rash, going over into exfoliative dermatitis with a duration of about 6—8 weeks before improvement. Four of them were mild cases where erythema and pruritus had totally disappeared after 2—3 weeks. The case with gastro-intestinal symptoms showed aphthae and a mild diarrhoea for some days. That with albuminuria showed this reaction for about two weeks but no pathological findings in the urine sediments.

No statistically significant difference was found — whether in the plasma gold level or in the urinary excretion of gold — between the cases with and without «toxic» reactions.

In Fig. 1 a typical curve of a case without «toxic» reaction is presented. Especially this case showed the highest value (0.41 mg per 100 ml) of gold level in plasma. Another case, which had been given Neosolganal in the same doses, showed the next high value (0.38 mg per 100 ml). This case, too, gave no «toxic» reaction. Among the cases that had been given Solganal B oleosum, the highest values of the plasma gold level were resp. 0.36 and 0.33 mg per 100 ml. These cases also showed no «toxic» reactions.

In one case (Fig. 2) the patient got a taste of metal in his mouth during the gold therapy. The interesting fact here was that this taste did not appear when the patient had his highest gold level in the plasma but later when this gold level had already diminished to below 0.08 mg per 100 ml. The gold concentration in mouth mucous membrane seems not to follow the plasma gold level, it seems to be a later accumulating effect there.

B.P.B. (w) b. 1894.

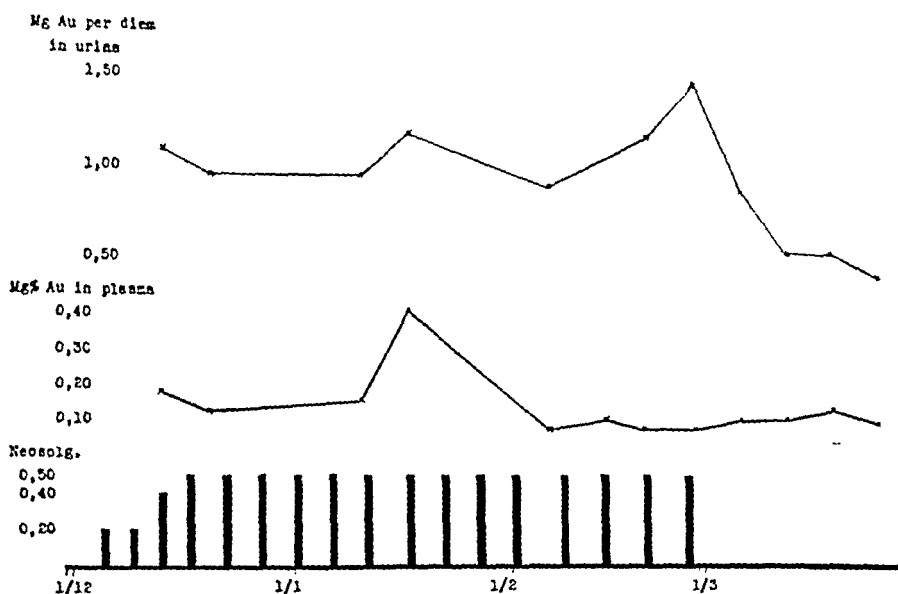


Fig. 1.

V.K.O.S (m) b. 1904.

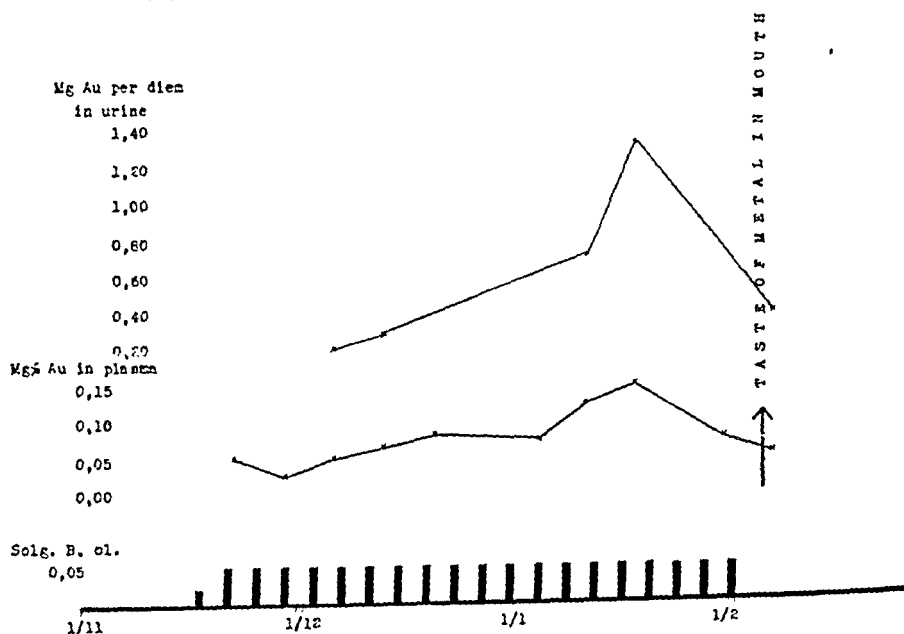


Fig. 2.

Fig. 3 illustrates a case with a mild «toxic» reaction. In that case the first «toxic» reaction appeared when the plasma gold level was at its highest point (0.22 mg per 100 ml). The symp-

N.S.N. (w) b. 1893.

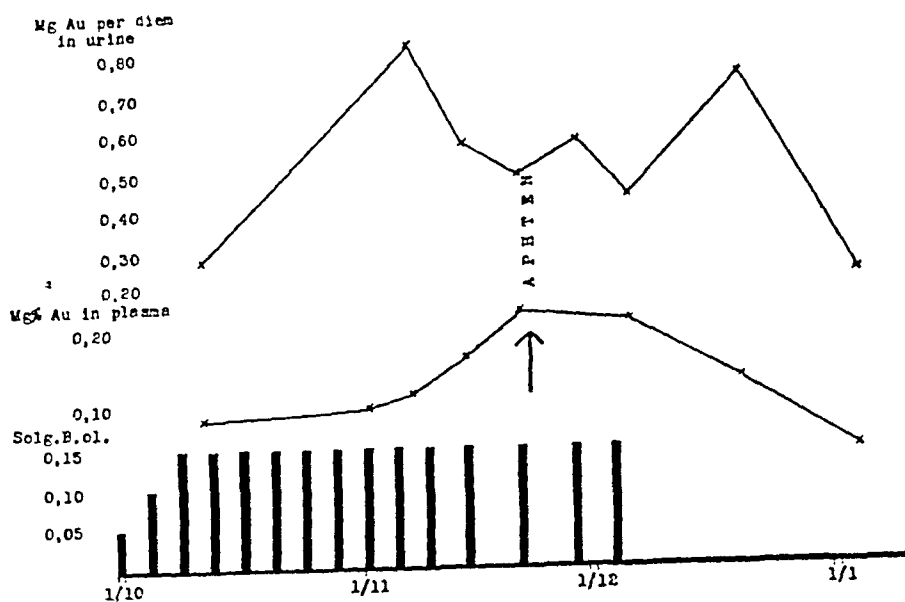


Fig. 3.

E.I.H.K. (w) b. 1913.

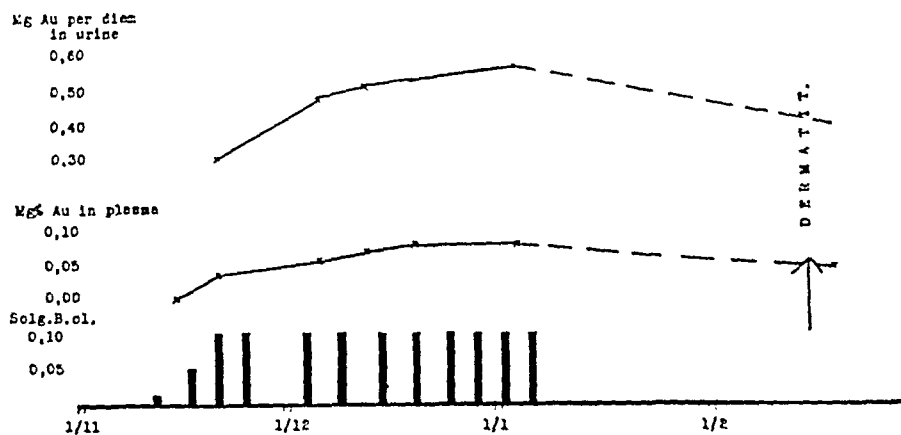


Fig. 4.

toms of aphthae and diarrhoea were rapidly transient in some days.

In Fig. 4 the case with the most severe «toxic» reaction of the whole series is presented. The first sign of «toxic» reaction here did not appear until six weeks after the last gold injection. In this case we got a very severe «toxic» reaction with exfoliative dermatitis that needed attendance at the Dermatological Depart-

ment for some weeks, before the patient, a female, improved. At the time the »toxic» reaction came the gold level in the plasma was low and the daily excretion in the urine also showed low values.

The other severe case with exfoliative dermatitis got her exanthema after she had been administered a total of 0.66 g Solganal B oleosum. In the middle of the cure intended, four days after the last injection of 0.10 g Solganal B oleosum — the injections were given at six days intervals —, the exanthema appeared when there were a plasma gold level of 0.05 mg per 100 ml and a normal concentration and excretion in the urine.

As regards the four remaining cases of dermatitis the plasma gold-level in two of them had been exceeding the highest values and were only resp. 0.04 and 0.05 mg per 100 ml at the beginning of the exanthema. In the other two this level was at the highest point, resp. 0.18 and 0.15 mg per 100 ml. Also in these cases the excretion was medium good. In these four cases the exanthema were more transient.

As seen, we have found no parallelism between the gold level in plasma and urine and the appearance of »toxic» reactions. Gold values of plasma and urine vary with the size and the intervals of the doses but are not directly proportional to this. Shortly after injection we find gold in plasma and urine and gold seems to enter very rapidly almost every cell of the body. The greatest concentrations, however, are found in the liver, spleen, kidneys and skin (Rosenberg, 1942). It can be that if we had had the possibility to follow the gold concentration in these organs, we could have found any more parallelism, but it is more probable that at bottom these »toxic» reactions are more an expression of a hypersensitiveness of the organism to gold than of real toxic origin.

Therefore we have no means of avoiding them. They may come when we least expect them. They may come if we use small doses of gold and if we use larger ones. Sundelin (1941) is of the opinion that large doses are no more toxic than small, though most observers do not agree to this (Comroe, 1944). According to Comroe (1944) and Hartung (1943), these reactions may occur following even small doses of gold, though they are usually less severe and of shorter duration than those which may follow large doses of gold salts. After our experience we agree with them.

Judging from the experience in our clinic also, a well-balanced high vitamin diet is of importance for avoiding a high per cent

of the thyreotropic anterior pituitary hormone. Attempts have been made with injections of the hormone into patients suffering from hypothyroidism, but the results have not been favourable. No increase of the secretion of the thyreoid can be produced by injection of thyreotropic hormone in these patients. The explanation is probably that the changes in the thyreoid gland in myxedema are so marked that the gland is in great part converted into fibrous tissue, and on fibrous scar tissue no parenchyma will grow. (Nielsen (77).) Moreover, the usual finding in myxedema is an increased content of thyreotropic hormone in the blood. This fact speaks against the theory of insufficient secretion of thyreotropic hormone as the cause of ordinary myxedema in man. Neither have there been found any signs of pituitary diseases on post mortem examination of cases with myxedema. Most often the pituitary is completely normal. In some cases small basophile adenomas have been found, but it is not proved that they are of any pathological significance for the hypothyroidism.

But still it is supposed that in certain cases diseases of the pituitary are able to cause hypothyreoidal conditions. A number of publications respecting myxedema in patients suffering from hypophyseal tumors have been issued (Leth Pedersen (78)). Transitional myxedema after trauma capitis (Jensen (79)) may also be the result of functional disturbances of the pituitary.

Snapper, Hunter et al. (80) have described a peculiar syndrome which they explain as a separate nosological unit, caused by pituitary disease. Characteristic for the syndrome is reduced basal metabolic rate, achylia, anemia, acrinia, hypogonadism and myelitic changes. They also saw excellent effects of a preparation containing thyreotropic anterior pituitary hormone. Some other authors, however, have denied that this syndrome has any connection with pituitary complaints and interpret it as being a peculiar form of spontaneous myxedema in adults, accompanied by anemia and assuming a pernicious character. The reason for the anterior pituitary extract having an effect should be its content of a certain amount of thyroxin. (Nielsen (77).) The question is not, however, settled, and the problems respecting the hypophyseal myxedema are still open to discussion.

The case which is to be discussed in the following pages will be of great interest as an illustration of the problems as to the pathogenesis of the diabetes mellitus and the hypothyreodism in man.

jection of 0.10 g of Myoral, pruritus appeared and the next day also exanthema, which in course of the following days spread out over the legs, trunk and the arms. Treatment with calcium i. v. and per os, combined with sodium thiosulphate i. v. had no effect. Then, on the third day after the exanthema had appeared, we began to give BAL i. m. 1.7 ml (2.5 mg per kg body weight in 10 per cent solution in peanut oil, containing 20 per cent benzyl benzoate) every fourth hour (7 times). Already after 2 injections the pruritus began to recede and after 6 injections the rash began to disappear. These injections were followed by two injections daily for a further nine days and during that period the rash entirely disappeared. The following course good. The patient discharged improved with very small remaining joint symptoms and capable for work a few weeks later.

The second case was a man of 52 years with a somewhat older rheumatoid arthritis (S. R. 10/1 h., AST neg., Agglutination pos.), who received in all 0.25 g of Myoral. Four days after the last i. m. injection of 0.10 g there appeared pruritus and rash. Before that he had been subfebrile for three days and had had focal reactions in his affected joints. Also here the dermatitis spread during the next few days out over trunk and extremities. Treatment with calcium i. v. and per os, combined with sodium thiosulphate i. v. had no effect. Then, on the third day after the appearance of the dermatitis, we gave BAL i. m. 1.9 ml (2.5 mg per kg body weight in the same 10 per cent solution) and this dose was repeated every fourth hour until it had been given seven times. In this case the pruritus was not so hardly severe as in the first case but ceased entirely first after the sixth injection had been given. The rash cleared up a little but not so much the first two days. Instead, we got a microscopic haematuria on the second day of BAL injections. Was it a reaction of the chrysotherapy or of the BAL? Or of the disease? We continued the BAL administration but reduced it to one injection daily. On the seventh day after initiation of the BAL administration the rash had entirely disappeared and simultaneously also the microscopic haematuria. The temperature was normal after the first day of BAL administration. — But the next day after finishing the BAL injections the rash and microscopic haematuria is coming back. We began again to give BAL i. m. in the same doses once per day during six days. During that period the rash and the microscopic haematuria again entirely disappeared. The following course good. Also this patient

discharged improved with small remaining symptoms and capable for work a few weeks later.¹

The injections were not painless. In all cases the patients were troubled by the first injections and we got infiltrations in gluteals, where the injections had been given. After 10—12 hours, however, these troubles receded at every point. During the cure these troubles also diminished so that there was very little such troubles at the end of it.

As seen, we have had good results in these five cases. We have used original BAL, the same as that used by Cohen et al. (1947), Lockie et al. (1947) and Ragan and Boots (1947).

It is encouraging that in BAL we have found a remedy, that seems to be of good effect in these often serious gold reactions, and this BAL therapy deserves further trial. But we must not forget that BAL also has toxic effects, and caution is recommended in its use.

Summary.

At present in our anti-infectious therapy of rheumatoid arthritis the treatment with gold salts plays the most important rôle.

¹ In the meantime before the proof in the clinic further three cases of gold-dermatitis were treated with BAL. All three were cases of rheumatoid arthritis. The first two of them had got Myoral (aurothioglucuronate), the third Aurothion (aurothiosulphate).

The first of these cases had got the last i. m. injection of 0.05 g Myoral on Aug. 9th (totally 0.50 g), pruritus and rash appeared about Oct. 15th, an interval of more than two months. In course of the following days exanthema spread out over the legs, trunk and the arms. Treatment with salves, calcium, sodium thioculphate i. v., had no effect. The exanthema exceeded to an exfoliative dermatitis. After one month in this state the patient was sent to the clinic Jan. 13th. BAL injections after scheme as in the first two cases were given 16th—25th. Already some hours after the first BAL injection the pruritus began to recede. After three days the rash cleared up and during the last days of BAL administration the rash entirely disappeared. The patient discharged Febr. 10th with nearly normal skin.

The second of the cases had got the last i. m. injection of 0.05 g Myoral (totally 0.30 g) Febr. 11th Febr. 18th aphthae in the mouth and 20 per cent eosinophili in the white corpuscles. Febr. 25th exanthema on the trunk. March 19th dermatitis. She was sent to the clinic April 1st, received BAL injections immediately after the scheme. The second day the pruritus began to recede and after four days the rash began to disappear. At the end of the injections the skin had been totally normal. Microscopic haematuria during some days.

In the third case the gold-injections had been given on the clinic. She had got i. v. injection of 2 ml aurothion April 30th and 4 ml May 4th. Four days later exanthema, pruritus and rash over trunk, the legs and the arms. Microscopic haematuria. Next day real dermatitis with severe pruritus. BAL administration was given after the scheme. After some hours the pruritus had receded and the next day the rash began to disappear. April, the 12th, the microscopic haematuria disappeared, the 14th the skin was totally normal and the BAL administration was interrupted.

However, gold salts are not the ideal drug because they are toxic, not all patients respond to them, and we often get recurrences after cessation of the chrysotherapy. The toxic reactions, which appear quite irregularly and capriciously, are the biggest crux. But are they really toxic?

Investigations of gold concentration in the plasma and urine during gold therapy in cases of rheumatoid arthritis with and without such reactions have indicated that there is no parallelism between these concentrations and the reactions in question. No statistically significant difference has been found, whether in the gold level of the plasma or in the excretion of gold in the urine, between the cases with and without »toxic» reactions. The reactions seem rather to be an expression of hypersensitiveness of the organism towards gold than to be of real toxic origin.

Therefore we have no means of avoiding them. They may come if we use small doses of gold and if we use larger ones. Those reactions following small doses, however, are usually less severe and of shorter duration. A well-balanced high vitamin diet is also of importance for avoiding a high per cent of such reactions.

Treatment of the gold reactions has taken a good step forward with the BAL therapy. Five cases, in which BAL has been used with good effect in the treatment of gold reactions are reported. The intramuscular injections have given local pains and infiltrations but no other symptoms of BAL toxicity are observed. The BAL therapy deserves further trial.

References.

- Cohen, Goldman and Dubbs: J. A. M. A. 133, 749, 1947. — Comroe: Arthritis and allied conditions. London 1944. — Edström: Acta Med. Scand. 103, 90, 1940. — Zt. Rheumat. 4, 233, 1941. — Eppinger: Die seröse Entzündung. Wien 1935. — Fischer and Wersig: Klin. W. 1936, 1079. — Freyberg, Block and Levey: J. clin. Invest. 20, 401, 1941. — Ann. Rheumat. Dis. 3, 77, 1942. — Gantzel and Larsen: Nord. Med. 19, 1473, 1943. — Harrestrup-Andersen and Normann: Nord. Med. 27, 1609, 1945. — Hartung: Bull. New York Acad. Med. 19, 693, 1943. — Lockie, Norcross and George: J. A. M. A. 133, 754, 1947. — Ragan and Boots: J. A. M. A. 133, 752, 1947. — Rinehart, Greenberg and Baker: Pract. Soc. exp. Biol. a. Med. 35, 347, 1936. — Rosenberg: Proc. Staff Meet. Mayo Clinic, 17, 264, 1942. — Secher: The treatment of rheumatic joint diseases. Copenhagen 1946. — Sundelin: Acta Med. Scand. Suppl. 117, 1941.
-

From the IVth Medical Service, St. Erik's Hospital, Stockholm.

The Effect of Tetraethylammonium Ion in Arteriosclerotic Heart Disease.

By

INGA LINDGREN and A. RUNE FRISK.

(Submitted for publication December 8, 1947.)

In preliminary reports on the effect and clinical use of tetraethylammonium ion Lyons and coworkers (3) state that among other types of visceral pain the pain in angina pectoris and coronary thrombosis is also relieved.

In order to evaluate the effect of tetraethylammonium upon pain in angina pectoris the following experiments were carried out.

Three carefully observed cases of proved angina pectoris were given tetraethylammonium bromide intravenously. These patients had previously been observed for a long time and were accustomed to various functional tests. After a test dose of 5 mg/kg of body weight and if no side-reactions or pronounced fall in blood pressure occurred the dose was doubled. A hypoxemia test under the influence of the drug was performed in two of these cases. Further details are given in the case-reports.

Case 1. J. E. A. (St. Erik's hospital No. 398/47), a fifty-nine-year-old male had angina pectoris of three years' duration. The attacks had increased in severity and frequency and came on even after slight exertion.

Physical examination revealed generalized arteriosclerosis and slight cardiac enlargement. The blood pressure was 185/125. The electrocardiogram showed normal rhythm rate 75, left axis deviation, depressed S—T 2, 3, 4 and flattened T. Hypoxemia test, February, 6, 1947: The patient breathed 10 per cent oxygen in 90 per cent nitrogen for 15

minutes, when typical anginal pain developed and the test was interrupted. The relative oxygen saturation of the arterial blood fell from 95 per cent to 79 per cent. Blood pressure rose from 155/105 to 195/125. According to Levy the test was positive with S—T changes in leads 1, 2 and 3.

Tetraethylammonium, 5 mg/kg of body weight or 370 mg intravenously, on February 26, produced a moderate drop in blood pressure, raised pulse rate, a disabling dyspnea and marked agitation. Simultaneously metallic taste on the tongue, sensation of dryness in the mouth, prickling sensation and warmth in hands and feet, dilatation of pupils and loss of accommodation occurred. After 30 minutes the patient complained of precordial pain, which persisted some hours in spite of nitroglycerine and oxygen administration. There were no changes in the EKG. The patient had previously been given daily injections of aminophylline and did not know he was given a new drug.

In order to exclude the possibility of a spontaneous attack having coincided with the injection the same dose was repeated a week later. This time the patient complained of tachycardia, shortness of breath and tightness in the chest, but the discomfort was slighter this time. The blood pressure fell from 175/110 to 145/95 in two minutes, and the pulse rate rose from 76 to 100 during this period. Two days later 10 mg/kg of body weight (740 mg) intravenously produced a drop in blood pressure from 160/105 to 115/80 and a rise in pulse rate from 66 to 96 within four minutes and the same slight symptoms as after the smaller dose occurred. Except for tachycardia, there were no EKG changes. March 3, a hypoxemia test was performed, 5 minutes after the intravenous injection of 10 mg/kg of body weight of the drug. The blood pressure, which after injection had fallen from 175/110 to 120/95, dropped to 100/70. The pulse rate, which after injection had risen from 76 to 96, in 10 minutes fell to 86. The relative oxygen saturation decreased from 96 per cent to 83 per cent. The test was finished after 20 minutes. The patient complained of some tightness in the chest and a severe headache. Oxygen given immediately afterwards raised the blood pressure to 150/110 in two minutes and the pulse rate fell to 66. The EKG showed T-wave inversion (positive T to negative T) in lead 1 five minutes after the injection. This effect on the EKG persisted for 45 minutes after the injection. During hypoxemia test there were no further EKG changes. Later T 1 rose again.

During the night the patient had repeated attacks of precordial pains with shortness of breath. He had never before had nocturnal pain.

Comment.

In this case of severe angina an attack of precordial pain occurred after the first injection of tetraethylammonium. The same dose, 5 mg/kg of body weight, was later repeatedly given with less pronounced symptoms. When the dose of the drug was doubled the subjective symptoms were about the same as after the

smaller dose. With the larger dose significant EKG changes, T-wave inversion, developed. Under the influence of the drug, however, the patient had no pain during 20 minutes of hypoxemia whereas hypoxemia of the same degree without previous injection of tetraethylammonium provoked pain after 15 minutes.

Case 2. S. S. P. (St. Erik's hospital No. 1125/47), a forty-six-year-old male had angina pectoris of 9 years' duration. The patient was completely invalidized by attacks even at rest, especially during the night.

Physical examination revealed obesity and generalized arteriosclerosis. The blood pressure was 140/90. EKG showed normal rhythm, pulse rate 60, left axis deviation, depressed S—T 1, 2, 4 and T 1, T 2, T 3, T 4 negative.

Repeated hypoxemia tests February, 26 and 27 induced typical anginal attacks after 14 and 17 minutes, respectively with S—T changes in leads 1, 2, 3, 4. The relative oxygen saturation fell from 92 to 68 per cent. Blood pressure dropped from 130/90 to 105/75, pulse rate rose from 64 to 85.

Tetraethylammoniumbromide, 5 mg/kg of body weight or 400 mg intravenously on March 4 produced drop in blood pressure from 105/75 to 90/65 and rise in pulse rate from 68 to 98 within two minutes. Simultaneously he got a severe headache, which subsided within one hour. Two hours after the injection the patient had a severe attack of anginal pain and headache.

The following day a dose of 10 mg/kg of body weight of 810 mg produced a maximum drop in blood pressure from 119/75 to 80/65 and a rise in pulse rate from 56 to 92 within three minutes. The patient got headache and a bursting sensation in the chest, but no anginal attack. The EKG showed a less negative T 1 and normalized T 2. The following night the patient had no attacks of pain.

Next day a hypoxemia test was performed 5 minutes after intravenous administration of 10 mg/kg of body weight of the drug. After 8 minutes the patient suddenly became grayish-white and the peripheral pulse could not be palpated. The oxygen saturation was then 66 per cent. The test was immediately stopped and oxygen was given. After 30 seconds the blood pressure was 90/70 and the pulse rate 68—70. The patient had no pain. There were no sequelae from this sudden drop in blood pressure and pulse rate.

This time T 1 was positive at the onset and became flattened after injection. During the hypoxemia no further EKG changes appeared.

Comment.

In this case of severe angina the administration of 5 mg/kg of body weight of tetraethylammonium was followed two hours later by a severe anginal attack. With the double dose a bursting sensation in the chest without pain occurred; the EKG was nor-

malized. A hypoxemia test under the influence of the drug provoked a severe collapse but no EKG changes.

Case 3. N. K. G. (St. Erik's hospital No. 473/47), a forty-three year old male with severe angina pectoris of three years' duration with attacks even at rest. The patient had some relief from daily injections of aminophylline.

Physical examination revealed marked arteriosclerosis for this age and slight cardiac enlargement. The blood pressure was 140/70.

The EKG showed normal rhythm, rate 75, left axis deviation, positive T 1, T 2, T 4 and negative T 3. Chest leads normal.

Tetraethylammonium, 5 mg/kg of body weight intravenously (420 mg) on March 4 produced a rise in pulse rate from 68 to 108 and a slight rise in blood pressure from 120/90 to 135/100 within two minutes. The double dose next day was followed by a rise in pulse rate from 68 to 116 and no changes in blood pressure (120/85). After 7 minutes the patient complained of severe pressure in the chest, the pulse rate was 110 and the blood pressure 130/100. This discomfort increased rapidly and he had an extremely severe anginal attack with nausea. Ten minutes after the injection the pulse rate was 108 and blood pressure 155/120. The attack subsided with oxygen and nitroglycerine treatment within 10 minutes but afterwards the patient felt very exhausted. Marked EKG changes occurred (fig. 1) with inversion of a positive T 1 and negative T 3 and S—T depression in leads 1 and 4. In spite of oxygen administration, pain and EKG changes persisted for 40 minutes after the injection.

Comment.

In this case of severe angina the administration of 10 mg/kg of body weight of tetraethylammonium provoked an extremely severe anginal attack with pronounced EKG changes.

The following case shows that myocardial infarction can occur following administration of tetraethylammonium.

A. S. M. (St. Erik's hospital No. 1917/47), a sixty-seven-year-old female with slight hyperthyroidism and hypertension of at least one year's duration. The patient had never had symptoms of angina pectoris. Physical examination revealed moderate arteriosclerosis and slight cardiac enlargement. The blood pressure 230/170 lowest, spontaneous blood pressure was 145/90 and the lowest pressure during an amytal test was 125/90. There was grade 2 retinopathy. B. M. R. was plus 32 per cent.

Tetraethylammoniumbromide, 5 mg/kg of body weight (310 mg) intravenously, produced a fall in blood pressure from 185/115 to 150/105 and a rise in pulse rate from 92 to 100 within one minute. No side-reactions occurred.

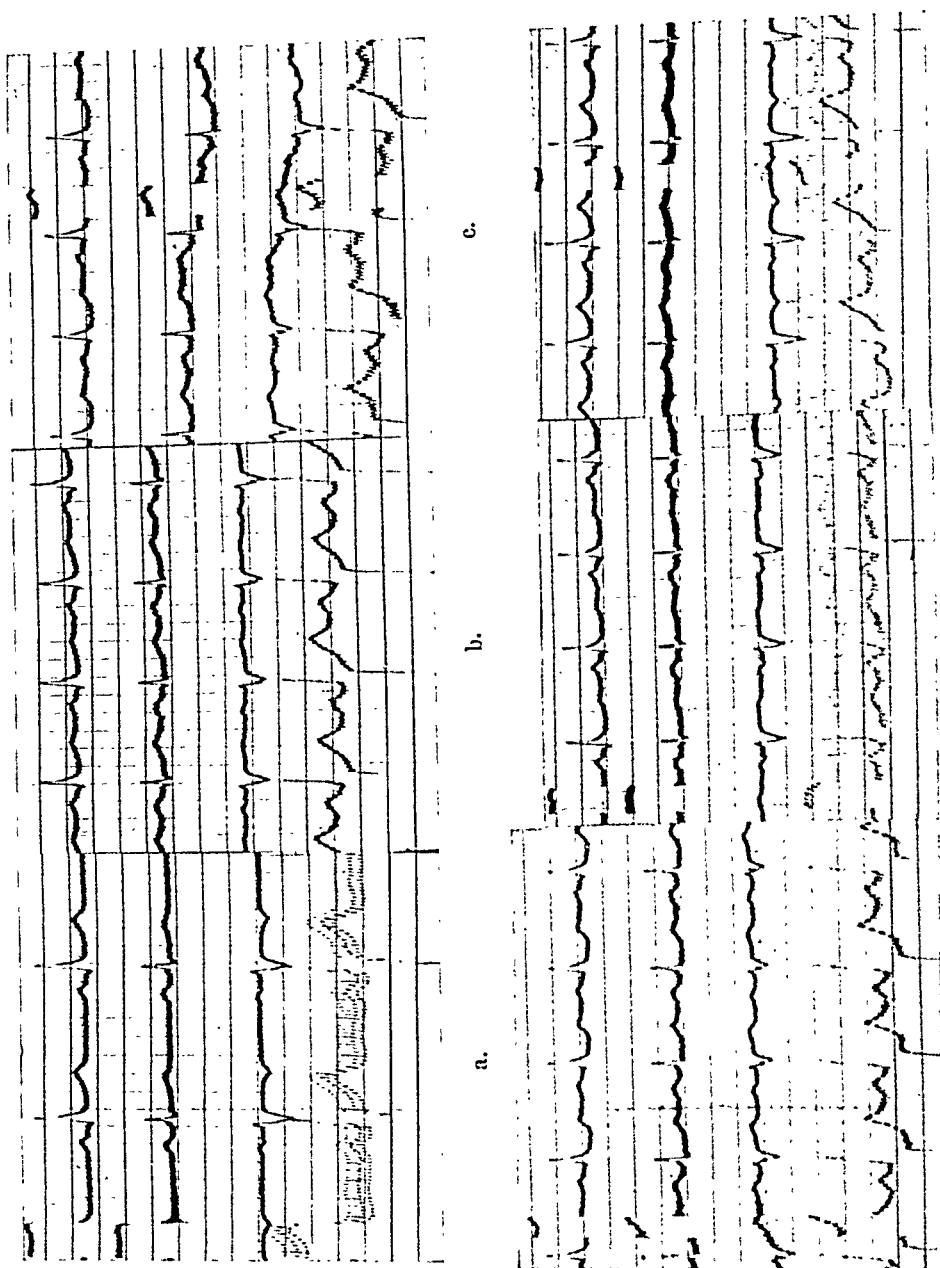


Fig. 1. EKG before and after injection of 10 mg/kg of body weight of tetraethylammonium in case 3.
 a) before the injection, b) 5 minutes, c) 10 minutes, d) 15 minutes, e) 20 minutes and f) 25 minutes after the injection.
 Pain started 7 minutes after the injection. Of interest is that EKG changes had occurred before pain started.

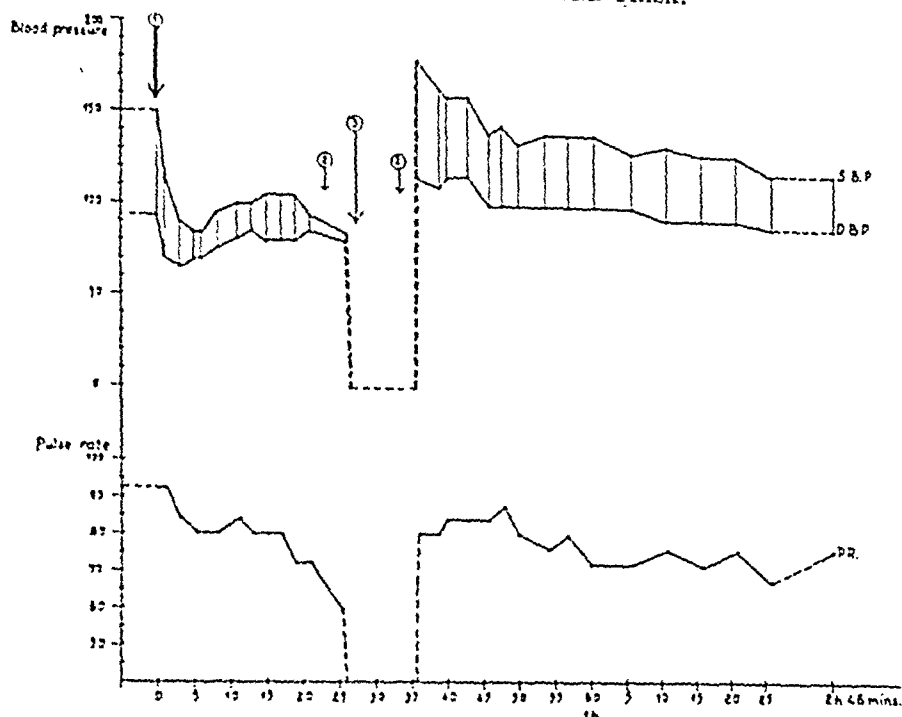


Fig. 2. S. B. P. — Systolic blood pressure. D. B. P. — Diastolic blood pressure.
P. R. — Pulse rate.

1. Intravenous injection of 10 mg/kg of body weight (625 mg) of tetraethylammonium.
2. Epinephrine intravenously and intramuscularly.
3. Oxygen administration.

After the double dose intravenously the blood pressure dropped within five minutes from 150/95 to 85/70 and simultaneously there was a fall in pulse rate from 92 to 80. During the next 20 minutes the patient felt sick and vomited. The blood pressure varied between 100/80 and 85/80 and the pulse rate declined to 60. Twenty-five minutes after the injection the patient collapsed. The peripheral pulse was not palpable. Epinephrine intramuscularly and intravenously as well as oxygen was administered and the patient was placed in Trendelenburg's position. Ten minutes later the pulse rate was 80 and the blood pressure 180/115. During the next five minutes, 40 minutes after the administration of the drug the patient had severe precordial pain. This pain remained unchanged in spite of morphine. During the next hour the blood pressure fell gradually to 120/80 and the pulse rate to 68. EKG showed posterior myocardial infarction. The patient had fever, over 38°C , for about 17 days. Blood pressure during the next month was about 140/90. Subsequent course has been uneventful. Fig. 2 shows the behaviour of blood pressure and pulse rate after the administration of the larger dose of the drug in this case.

Comment.

This case of hypertension showed a moderate drop in blood pressure and only a slight rise in the pulse rate when 5 mg/kg of body weight of tetraethylammonium was given intravenously. The double dose provoked a rapid fall in blood pressure and simultaneously a fall in the pulse rate. Twenty-five minutes after the injection she collapsed and developed an acute myocardial infarction.

Summary.

The effect of the administration of tetraethylammonium in these cases of severe angina pectoris has been studied in detail. In addition, one case of myocardial infarction after injection of this drug is reported. In our experience, this drug can be dangerous in angina pectoris and in hypertension in patients with marked generalized arteriosclerosis. The importance and necessity of great precaution in the administration of tetraethylammonium to such patients is evident.

References.

- Berry, R. L., Campbell, K. N., Lyons, R. H., Moe, G. K., and Sutler, M. R.: *Surgery* 20; 525; 1946. — Coller, F. A., Campbell, K. N., Berry, R. E. L., Sutler, M. R., Lyons, R. H., and Moe, G. K.: *Ann. of Surg.* 125; 729; 1947. — Lyons, R. H., Moe, G. K., Neligh, R. B., Noobler, S. W., Campbell, K. N., Berry, R. L. and Rennick, B. R.: *Am. J. Med. Sci.* 213; 315; 1947. — Larsson, Y., and Frisk, A. R.: *Acta med. Scand. Suppl.* 196; 212; 1947. — Lindgren, I., and Olivecrona, H.: *J. of Neurosurg.* 4; 19; 1947.
-

From the Biochemical Institute, Aarhus University (Chief: Professor Fritz Schönheyder, M. D.), from the Medical-epidemic Department, Aarhus Marselisborg Hospital (Chief Physician: Gregers Nørby, M. D.), and from the Medical Department of Aarhus County Hospital (Chief Physician: Professor Aage Th. B. Jacobsen, M. D.). (Denmark.)

On Serum Copper in Angina Simplex and in Infectious Mononucleosis.

By

SVEN MUNCH-PETERSEN.¹

(Submitted for publication December 8, 1947.)

During the work with serum copper under normal and pathologic conditions a striking difference was found between the values for patients with angina simplex (used as collective designation of different forms of more unspecific sore throat) and with infectious mononucleosis, the increase which occurs in these affections being considerably larger in the latter than in the former. This appears clearly, when the average of the 2 first values, which are observed in each case of illness is compared with a control material, as done below. I have failed to find a report of this phenomenon in the literature.

Method and Cases.

For the determination of serum copper was employed the modification of the sodium diethylcarbamate method given by Levin Nielsen (1, 1944), this method being the most sensitive of the colorimetric methods indicated for copper. Serum was destroyed by wet ashing with concentrated acids and the iron bound by sodium pyrophosphate at alkaline reaction. The coloured compound which is formed by the addition of carbamate is extracted with isoamylalcohol. The colorimetric determination takes place

¹ Aarhus, Denmark.

Probit Curve
Normal Values

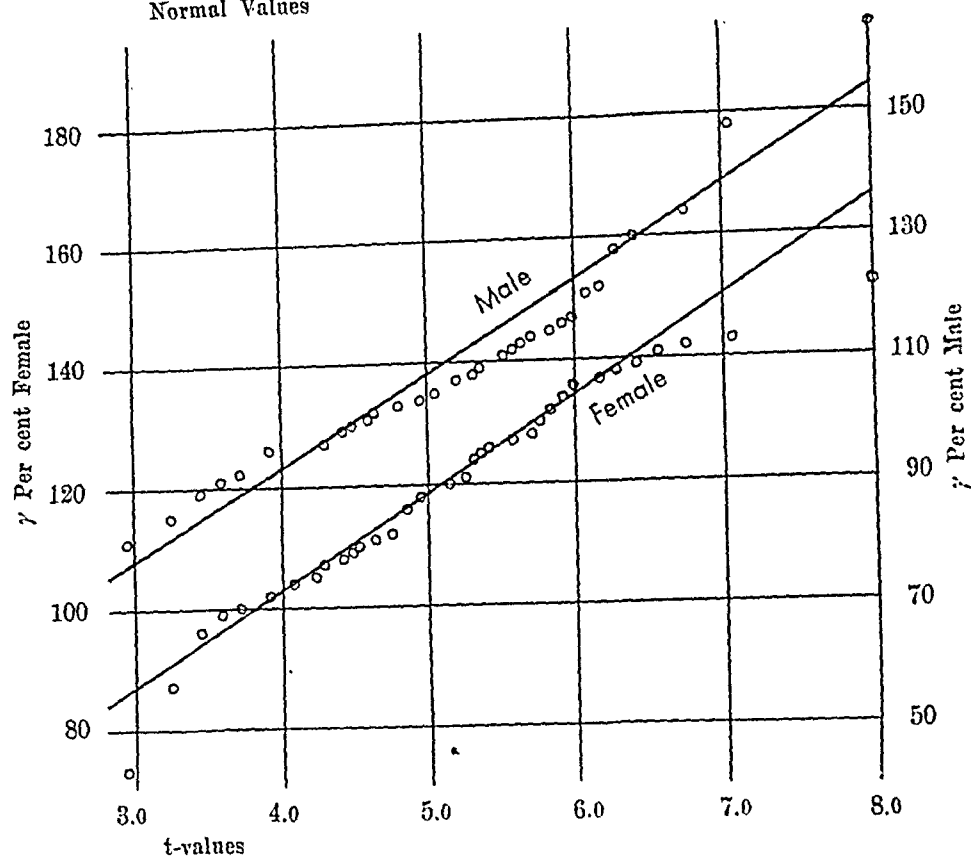


Fig. 1.

in microcuvettes in a Pulfrich photometer. Duplicate determinations were carried out with a maximum difference of 10 %, the blank value from the reagents in each case being subtracted.

The normal material, which has been plotted according to the probit method in Fig. 1, consists of 50 women and 50 men. The following values were found: average value for women $118 \gamma \% \pm 2.2 \%$, standard deviation $\sigma = 15.7$ (range 73–152 $\gamma \%$), average value for men: $108 \gamma \% \pm 2.1 \%$, $\sigma = 15.2$ (range 81–164 $\gamma \%$). Levin Nielsen (2, 1944) has found the following average values for 100 women and 30 men: women $123.15 \gamma \%$, $\sigma = 16.24$; men $110.53 \gamma \%$, $\sigma = 12.14$. Both the material of Levin Nielsen and the author's own material show, that there is a significant difference between the average values for women and men. There is good agreement between the two materials. Levin Nielsen's blood samples have been drawn in the forenoon after breakfast. My samples were drawn in the morning from fasting persons.

The blood samples from my patients are, contrary to my own normal material, but on an analogy with Levin Nielsen's drawn in the forenoon 1—2 hours after breakfast, the first one generally the day after admission. The first sample from patients with angina simplex was thus taken 2—4 days after the appearance of the first symptoms. The first blood sample from patients with infectious mononucleosis was taken on the 8th to 17th day after the beginning of the affection, the sore throat in this disease developing more slowly, causing the patients to be hospitalized later.

The investigation in this work comprises in all 32 patients, 29 from the Medical-epidemic Department of Aarhus Marselisborg Hospital and 3 from the Medical Department of Aarhus County Hospital. It consists of 16 women and 6 men with angina simplex and of 3 women and 7 men with infectious mononucleosis. In none of these patients any affection or complication was found which might be supposed to influence the serum copper. The essential treatment has been confinement to bed.

From the material were excluded 4 patients on whom only one examination was carried out. In addition 2 children aged 11 and 12 with infectious mononucleosis were omitted, the average of serum copper in normal children in this age group being somewhat higher than in adults. In 8 normal children aged 11 I thus found an average of $152 \gamma \% \pm 1.4 \%$ (range 131—166) and in 11 normal children aged 12 correspondingly $134 \gamma \% \pm 17.5 \%$ (range 107—169). On the other hand, serum copper values from 14 years and upwards seem to lie at the same level as in adults. In 4 normal children aged 14 the average of serum copper was for instance $118 \gamma \% \pm 22.2 \%$ (range 99—145).

Almost all the patients had distinct symptoms of throat inflammation at admission. Bunnell's reaction was not performed on patients with angina simplex, this test only being carried out when the differential blood count had given suspicion of infectious mononucleosis. In 2 cases of infectious mononucleosis the reaction was not performed, and in one case it was negative in spite of the blood picture being typical of infectious mononucleosis.

Simultaneously with the drawing of blood samples for serum copper determination, the blood picture and blood sedimentation rate were examined.

Results.

The results of copper analyses, differential blood count, blood sedimentation rate, Bunnell's reaction, the temperature measured on the morning of the day of examination, and the day since the beginning of the illness are to be found in Table 1. (As previously mentioned the material consists of 22 cases with angina simplex and 10 cases with infectious mononucleosis.)

It appears directly from the serum copper values given for patients with angina simplex as well as infectious mononucleosis that an increase occurs early in the illness, this increase often being accentuated from the first to the second value, after which the serum copper gradually returns to the normal level.

As an expression of serum copper in an early stage of the illness it was therefore determined to use the average of the two first values. This was chosen although the highest value in a few cases is not included in the mean.

It is seen from Table 2, that the 4 means for patients with throat diseases are considerably enhanced compared with the average values of the normal material. In a normal material it is reasonable to assume that there will only quite exceptionally be values whose deviation from the average exceeds twice the standard deviation, which may be put at 15 in this case. All the averages found for patients, men and women respectively, with angina simplex and infectious mononucleosis are enhanced over the average values to such an extent that a statistically certain elevation may be assumed.

Besides, it appears from Tables 1 and 2 that a larger elevation has occurred in cases with infectious mononucleosis than in cases with angina simplex. Although the material only comprises 3 women with infectious mononucleosis it is seen, that the difference between the average values of these patients and the women with angina simplex amounts to a little more than twice the error of the difference; this agrees with the 95 percent limit according to Student's distribution. In the case of the men the difference between the averages is distinctly significant.

As mentioned above the blood samples from cases with infectious mononucleosis have been taken somewhat later in the illness. This however, does not play a rôle when comparing the two diseases, as it is seen from Table 1, that the highest value of serum

Table 1.

No.	Sex.	Age	Diagnosis	Days	S. R. mm/1 hour	Leukocytes		Temp. °C	Serum copper
						Count.	Per cent of mono- nuclear cells		
1	Female	32	Angina simplex	9	43	9 160	22	39.7	146
"	"	"	"	12	75	14 300	27	37.6	159
2	Female	22	Angina simplex	4	58	18 600	22	38.7	179
"	"	"	"	7	42	7 880	32	36.3	200
"	"	"	"	11	21	6 400	36	36.4	143
3	Female	33	Angina phlegmonosa	5	50	8 800	16	39.7	161
"	"	"	"	8	109	8 960	30	37.8	194
4	Female	24	Angina simplex	4	25	9 920	32	37.8	153
"	"	"	"	8	18	7 080	—	37.0	168
"	"	"	"	11	15	10 320	37	37.0	160
5	Female	31	Angina simplex.	4	57	12 080	33	38.5	156
"	"	"	"	9	80	8 880	25	37.2	184
"	"	"	"	12	56	5 000	45	36.0	180
"	"	"	"	20	15	8 520	42	(amb)	137
"	"	"	"	44	5	3 880	40	(amb)	133
6	Female	19	Angina simplex	2	15	8 800	24	38.2	126
"	"	"	"	5	10	3 080	40	36.5	147
"	"	"	"	8	6	5 200	41	36.4	128
"	"	"	"	18	6	8 440	30	(amb)	129
7	Female	36	Angina phlegmonosa	4	92	13 520	27	39.1	175
"	"	"	"	7	65	9 760	31	37.2	185
"	"	"	"	10	51	8 760	27	36.7	184
"	"	"	"	16	—	6 120	40	36.8	149
"	"	"	"	25	20	6 000	42	(amb)	146
"	"	"	"	32	17	8 000	36	(amb)	123
8	Female	41	Angina simplex	15	25	—	28	39.5	146
"	"	"	"	20	12	6 040	24	36.7	161
"	"	"	"	29	23	7 400	19	(amb)	159
"	"	"	"	35	15	8 720	28	(amb)	143
9	Female	52	Angina phlegmonosa	7	105	—	21	38.3	205
"	"	"	"	11	55	8 600	29	37.2	198
"	"	"	"	22	83	7 560	38	(amb)	186
"	"	"	"	27	59	9 160	34	(amb)	149
10	Female	19	Angina simplex	4	55	6 440	—	37.2	161
"	"	"	"	7	24	7 600	—	37.1	137
"	"	"	"	17	20	6 200	—	(amb)	149
"	"	"	"	22	15	5 600	31	(amb)	119
11	Female	45	Angina simplex	2	34	15 760	—	37.3	128
"	"	"	"	5	22	6 800	46	36.3	137
"	"	"	"	12	9	6 800	38	(amb)	124
"	"	"	"	20	17	6 400	32	(amb)	112
12	Female	38	Angina simplex	6	68	8 000	32	37.3	192
"	"	"	"	9	25	6 800	26	37.0	166
"	"	"	"	12	50	8 200	18	36.7	178
"	"	"	"	18	43	6 600	29	(amb)	153
"	"	"	"	25	19	3 840	43	(amb)	157
"	"	"	"	39	11	5 200	30	(amb)	135
13	Female	15	Angina simplex	6	3	8 000	44	37.8	208
"	"	"	"	10	10	6 400	—	36.6	185
"	"	"	"	13	11	5 200	47	(amb)	180
"	"	"	"	20	5	4 800	48	(amb)	162

Table 1. (Cont.)

No.	Sex.	Age	Diagnosis	Days	S. R. mm/1 hour	Leukocytes		Temp. °C	Serum copper
						Count.	Per cent of mono- nuclear cells		
13	Female	15	Angina simplex	27	3	7 400	47	(amb)	143
14	Female	31	Angina simplex	3	34	11 400	22	37.9	146
"	"	"	"	6	—	7 400	—	36.5	173
"	"	"	"	8	42	7 440	26	36.3	149
15	Female	14	Angina phlegmonosa	9	—	13 240	23	38.6	195
"	"	"	"	12	—	6 640	22	36.8	184
"	"	"	"	15	17	6 600	30	36.6	157
16	Female	17	Angina simplex	3	33	6 000	20	37.1	148
"	"	"	"	6	—	4 560	23	36.3	161
"	"	"	"	10	19	6 600	44	36.1	148
"	"	"	"	19	9	6 600	42	(amb)	130
"	"	"	"	26	9	4 800	39	(amb)	140
17	Male	41	Angina simplex	2	49	11 040	19	38.2	145
"	"	"	"	4	61	13 600	20	37.2	154
18	Male	21	Angina ulcerosa	5	30	8 760	30	36.8	157
"	"	"	"	9	22	7 500	31	36.3	140
19	Male	24	Angina simplex	4	22	—	46	38.1	149
"	"	"	"	7	19	4 800	44	37.2	164
"	"	"	"	10	—	5 200	34	37.2	148
"	"	"	"	15	14	5 800	36	37.1	136
"	"	"	"	29	6	6 080	36	(amb)	143
"	"	"	"	43	8	6 000	36	(amb)	117
20	Male	20	Angina phlegmonosa	9	66	20 480	22	38.6	156
"	"	"	"	12	38	9 860	26	37.0	149
21	Male	20	Angina simplex	4	25	6 800	29	37.0	138
"	"	"	"	7	7	5 800	17	36.7	139
"	"	"	"	10	12	9 200	24	(amb)	138
"	"	"	"	20	5	6 000	35	(amb)	145
22	Male	19	Angina simplex	8	24	8 000	21	37.2	166
"	"	"	"	11	11	8 400	34	36.6	151
"	"	"	"	33	5	6 000	38	(amb)	132
23	Female	17	Mononuel. infect. (+Bunnell's test)	17	18	9 200	66	36.6	178
"	"	"	"	20	13	6 400	76	36.8	177
"	"	"	"	23	11	6 000	59	36.5	193
24	Female	17	Mononuel. infect. (+Bunnell's test)	18	11	6 400	73	36.6	175
"	"	"	"	21	15	4 680	72	36.8	165
"	"	"	"	39	7	5 560	39	(amb)	112
25	Female	22	Mononuel. infect. (+Bunnell's test)	15	32	7 800	50	38.7	282
"	"	"	"	19	45	6 000	45	37.8	256
"	"	"	"	22	—	8 000	51	37.2	241
"	"	"	"	28	19	5 200	57	36.7	232
"	"	"	"	42	—	5 080	44	(amb)	237
26	Male	20	Mononuel. infect. (Bunnell's test: not performed)	47	29	4 760	33	(amb)	215
"	"	"	"	10	25	8 320	51	38.3	182
"	"	"	"	14	20	—	83	36.6	147
"	"	"	"	25	7	6 480	30	(amb)	124

Table 1. (Cont.)

No.	Sex.	Age	Diagnosis	Days	S. R. mm/1 hour	Leukocytes		Temp. °C	Serum copper
						Count.	Per cent of mono- nuclear cells		
27	Male	30	Mononuel. infect. Bunnell's test: neg- ative, not repeated)	28	10	10 360	56	37.7	201
"	"	"	"	31	13	8 760	51	37.3	204
"	"	"	"	34	8	8 680	40	36.9	185
"	"	"	"	41	7	8 560	53	(amb)	170
"	"	"	"	58	—	4 000	40	(amb)	152
"	"	"	"	68	—	—	—	(amb)	124
28	Male	23	Mononuel. infect. (+Bunnell's test)	10	50	11 040	51	39.4	226
"	"	"	"	14	40	7 880	55	36.7	214
"	"	"	"	17	50	8 720	45	37.0	176
"	"	"	"	27	28	5 600	30	(amb)	151
29	Male	14	Mononuel. infect. (+Bunnell's test)	11	16	10 640	87	38.6	193
"	"	"	"	14	30	12 800	68	37.4	199
"	"	"	"	17	16	7 240	79	36.7	191
30	Male	19	Mononuel. infect. (Bunnell's test: not performed)	12	11	15 760	63	38.0	177
"	"	"	"	14	19	14 520	76	37.4	196
"	"	"	"	26	6	5 200	53	(amb)	156
"	"	"	"	40	4	4 120	43	(amb)	136
31	Male	19	Mononuel. infect. (+Bunnell's test)	15	18	10 840	57	38.4	168
"	"	"	"	18	7	7 880	86	36.6	178
"	"	"	"	22	6	6 280	75	36.6	142
"	"	"	"	36	2	4 840	33	(amb)	146
"	"	"	"	44	4	6 200	48	(amb)	121
32	Male	23	Mononuel. infect. (+Bunnell's test)	5	32	13 400	50	38.4	190
"	"	"	"	8	8	7 200	58	36.9	175
"	"	"	"	15	7	7 660	69	(amb)	171
"	"	"	"	22	4	9 840	66	(amb)	162
"	"	"	"	31	5	6 480	47	(amb)	123

copper (generally in connection with the second blood sample) is almost always included in the average values which form the basis of the judgment.

The blood sedimentation rate is somewhat higher in angina simplex than in infectious mononucleosis, in agreement with previous findings. The serum copper values conversely are greater in the last mentioned affection. In one and the same patient the serum copper and blood sedimentation rate are diminished as the illness proceeds, but in general it is not possible to establish

Table 2.

Serum Copper Values in Normal Humans and in Patients with Throat Diseases.

		Female	Male
Normal subjects	number	50	50
	aver. value	119 ± 15.7	108 ± 15.2
	range	73-152	81-164
Subjects with angina simplex	number	16	6
	aver. value	168 ± 21.0	151 ± 7.1
	range	133-202	139-159
	dev. from aver. value	+49	+43
Subjects with infectious mononucleosis	number	3	7
	aver. value	206 ± 55.0	190 ± 18.6
	range	170-269	165-220
	dev. from aver. value	+87	+82

a closer relation between serum copper and sedimentation rate on the basis of the present material. In a few cases the sedimentation rate, however, follows the increase in serum copper from the first to the second blood sample. No relationship was found between serum copper and morning temperature, nor between serum copper and leukocyte count.

In a few of the patients with angina simplex and infectious mononucleosis during the whole course of the illness, simultaneously with serum copper determinations analyses for copper were performed on the coagulum remaining in the glass after vigorous centrifuging and complete pipetting off of the serum. In this way one has been able to judge the copper content of the red blood corpuscles. The values observed for the copper content in the blood coagulum were all, independently of the serum copper values, around 100 γ %, which is only slightly higher than in normal persons. Consequently the rise in blood copper values observed in patients with sore throat occurs almost entirely in the serum. Holmberg (3, 1941) has related augmented values of the copper content in the blood of pregnant women. This increase also is mainly due to a rise in the copper content of the serum.

At the present moment it is difficult to explain the increase of serum copper in angina simplex and infectious mononucleosis. It must be emphasized that the increase observed in infectious mononucleosis is very large, as large as the maximum increase

demonstrated in any illness. This has apparently not been reported previously. Only in exudative progressive forms of tuberculosis of the lungs and in polyarthritis (Heilmeyer (4) and Van Ravesteijn (5)) an elevation of the same order of magnitude has been observed.

Summary.

Investigations of serum copper in 22 patients with angina simplex and 10 patients with infectious mononucleosis showed an increase of serum copper in both groups. The increase which occurs is considerably larger in the latter than in the former.

I am indebted to the »Nordisk Insulinfond» for a grant received in aid of my work on serum copper.

Literature.

- 1) Nielsen, A. Levin: *Acta physiol. Scand.* 7, 271, 1944. — 2) Nielsen, A. Levin: *Acta med. Scand.* 108, 87, 1944. — 3) Holmberg, C. G.: *Acta physiol. Scand.* 2, 71, 1941. — 4) Heilmeyer, L., W. Keiderling, and G. Stüwe: *Kupfer und Eisen als Körpereigene Wirkstoffe*. Jena. 1941. — 5) Van Ravesteijn, A. H.: *Koperstofwisseling bij Den Mensch*. Diss. Utrecht. 1945.
-

From Maria Hospital, Medical Department, Helsingfors.
(Head: Prof. F. Saltzman.)

On Auricular Fibrillation and Block, in Connection with a Recent Case.

By

GÖSTA BJÖRKENHEIM.¹

(Submitted for publication January 14, 1948.)

In cases of auricular fibrillation, a certain functional block of the auriculo-ventricular conduction is usually present: only few of impulses reach the ventricles outside the refractory period, and the ventricles beat irregularly. If a real auriculo-ventricular dissociation appears, the case is entirely different. In experiments published in 1904 Frédéricq provoked auricular fibrillation with irregular ventricular rhythm on the exposed heart of a dog, by means of faradaic irritation. When the auriculo-ventricular bundle was cut off or squeezed in a tourniquet, the ventricular rhythm became regular, while the auricles still fibrillated. Complete auriculo-ventricular block in the presence of auricular fibrillation, *i. e.* regular ventricular rhythm, was clinically observed for the first time by Kahn and Münzer, while Lewis and Mack were the first to describe a case of auriculo-ventricular block with supervening fibrillation. Since then, several authors have paid attention to this phenomenon (among others Wenckebach and Winterberg, Scherf and Boyd, Rasmussen). While fibrillation and complete auriculo-ventricular block seem to combine quite frequently, flutter with complete block is very rare. In Scandinavian medical literature, a paper by Thorborg quotes 31 published cases of this kind, to which he adds 3 out of his own experience. Further, he describes 4 cases of fibrillation with complete auriculo-ventricular block.

¹ Sandvikskajen 11 A, Helsingfors.

In cases of bundle branch block, auricular fibrillation is apparently rather frequent. Yater has edited a collocation of published cases from various authors, where fibrillation appears in 114 cases among 829 bundle branch block patients (14 %). Müller has found a family where the father and 3 of his children, aged 28—42, showed symptoms of mitral disease with fibrillation, bundle branch block and irregular ventricular rhythm. In most of Thorborg's cases as well, the electrocardiogram showed signs of disturbance of the intra-ventricular conduction.

At Maria Hospital, a patient with auricular fibrillation and block has been treated recently, and I shall now give a short account of the course of her disease. (She has also received treatment at the Stengård Hospital, head Prof. P. Soisalo.)

Pat. E. R. ♀, clerk, b. 1891. Father and several paternal relations dead before the age of 50, from heart disease. In childhood diphtheria, scarlatina, etc., without complications. Frequent soreness of throat, no serious angina, no disease of the joints. Sometimes migraine-like headache. At the age of 18, goitre was established. No diseases of the lungs; husband died of tub. pulm. Menses normal. 4 children, one miscarriage. No signs of venereal infection. Because of weariness, thinness and occasional irregularities of the heart functions, she had a months hospital treatment in 1933. Thyroid gland slightly enlarged, traces of tremor. Basal metabolism + 16 % (?). Lungs normal, with the exception of a calcareous shadow in the right hilus. She harboured a fish tapeworm that was expelled by filicin. ESR 24/54. Heart size ordinary, arcus aortae slightly prominent. Singing systolic murmur at the apex, P II accentuated (?). Blood pressure 145/90. Pulse occasionally irregular, 48—70/min. Electrocardiogram: Rhythm slightly irregular 64/min. Ventricular extrasystoles. P low, PQ 0.31", QRS-complexes normal. S—T iso-electric and T positive in all leads. She was treated with sedatives, discharged as improved with the diagnosis Myodegen. cordis. In 1936, another month in hospital for similar troubles. Small knob in the thyroid gland. Basal metabolism polyclinically + 23 %, in hospital + 6 %. Coli bacteria in the urine, subfebrile. Slight left enlargement of the heart, retrocardial space moderately narrowed. Systolic murmur, ictus heaving. Arteria radialis normal. Pulse irregular and uneven, frequency 50—88/min. Blood pressure 150/100. Diagnosis Struma nodosa. Pyelitis ac. In 1942 she asked medical advice for tiredness and thinning: high blood pressure and sparse pulse were established. In January 1943, she had an attack (Adams-Stokes' ?) and was taken into hospital for a couple of months. On arriving, somewhat exhausted, no oedemas. Thyroid gland ordinary. Eyes ordinary. Heart size ordinary, tones dull, systolic murmur at the aorta. Pulse irregular, 26—64/min, mainly below 50. Blood pressure 150/90. Electrocardiogram: Rhythm irregular, about 40/min. No P-waves, QRS-complexes low, < 0.10". S—T iso-

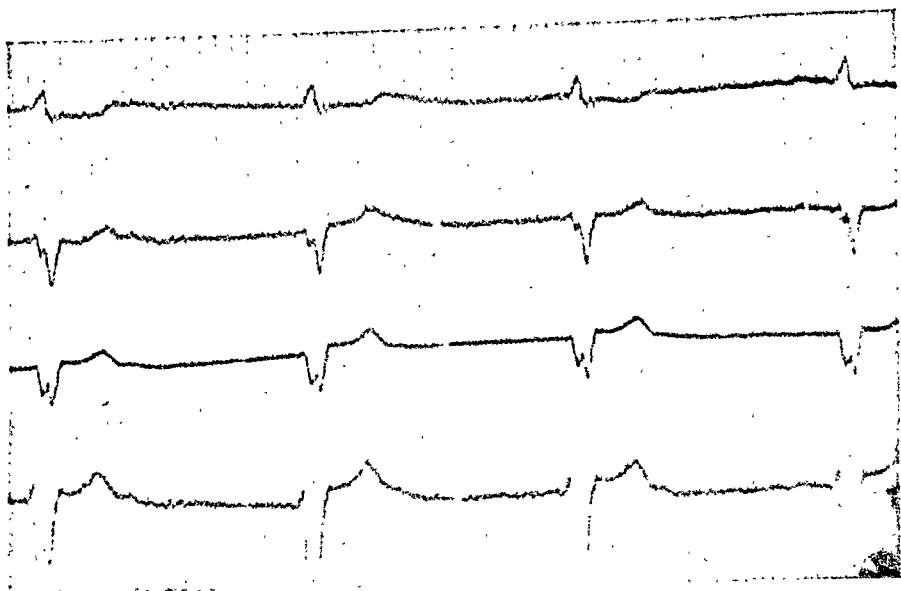


Fig. 1.

electric and T positive in all leads. Received sedatives and stimulants, and was also treated for angina and polyarthritis occurring during her stay in hospital. Diagnosis Myodegen. cordis. After discharging back to work. Later on, difficulty in breathing, swollen legs, no palpitation of the heart, nor pain in the chest. In addition prolapsus of the genitals. In Spring 1947, again one month in hospital. Thyroid gland ordinary, moderate anaemia. Slight pulmonary congestion. Liver edge 3 fingerbreadths below costal bow. Arteria radialis straight, firm, pulse regular 32—70/min. Blood pressure 130/70, later 135/90. Heart considerably enlarged to right and left, retrocardial space moderately narrowed. Aorta large, aortic knob prominent. Tones dull, no sonorousness, strong systolic murmur all over the heart. Electrocardiogram (fig. 1): Rhythm regular, about 36/min. No P-waves, QRS-complexes broad (0.16"), split, greatest deviation positive in I and negative in remaining leads. S—T lowered in I, iso-electric in II, raised in III and precordial leads. T positive in all leads. The patient was treated with coffeeine and ferrum. Oedemas vanished, liver size diminished. Discharged as improved, with the diagnosis Insuff. valv. mitralis. Fibrillatio atriorum. Bundle branch block. Anemia simplex.

In a middle-aged woman with indefinite symptoms of hyperthyreosis, a first degree heart block is found. Subsequently, auricular fibrillation with remarkably slow rhythm is developed. After this, the heart is greatly enlarged, the sparse and irregular pulse becomes regular. The electrocardiogram shows ventricular com-

plexes typical of left bundle branch block. Simultaneously, symptoms of a slight circulatory failure appear but diminish without digitalis treatment. The development indicates that the auriculo-ventricular block is successively aggravated.

As mentioned above, the combination of auricular fibrillation and block is described by several authors and is surely quite frequent, fibrillation as well as block being usual symptoms of heart diseases. Aetiologically, these combined cases are divided by Thorborg into two groups: 1. Spontaneous cases, where the heart disease in question leads to auricular fibrillation as well as to block, and 2. Cases, caused by medicines. In two of Thorborg's cases, digitalis treatment of auricular fibrillation led to complete auriculo-ventricular block, which vanished when the digitalis medication ceased.

The diagnosis may be made by observing how the irregular rhythm of the auricular fibrillation grows even and slow. As a rule, a definite diagnosis is only electrocardiographically obtained. When auricular fibrillation combines with bundle branch block, the ventricular rhythm is not always regular, in Müllers 4 cases arrhythmia was established. In my case, it might be supposed that the auriculo-ventricular block has become complete and is thus responsible for the regular rhythm. Then, the new automatic centre should be situated in one of the branches, probably the right one. It might also be imagined that the centre is in the unbranched bundle of His, below the block, and that actually a left side retardation co-incides. During the four years that have elapsed since the latest electrocardiogram showing normal ventricular complexes was taken, also the considerable enlargement of the heart has occurred.

According to Wenckebach and Winterberg, it is of little importance to the heart functions if auricular fibrillation is complicated by complete block. Consequently, the therapy ought to be the same as in uncomplicated cases. Digitalis treatment of these patients is of course not contra-indicated, except in cases where digitalis has caused the complete block. Gager describes a case where digitalis therapy had no effect, while after quinidine treatment the block vanished and sinus rhythm appeared. In connection with a case published by Kerr and Bender, de Boer warns against quinidine therapy in this state as easily leading to ventricular fibrillation.

Summary.

Report of a case where, in a middle-aged woman, occurs a first degree auriculo-ventricular block, subsequently auricular fibrillation, and finally regular bradycardia with electrocardiogram typical of bundle branch block. When auricular fibrillation combines with bundle branch block, the fibrillatory arrhythmia may continue, and it is suggested that in this case a complete auriculo-ventricular block should have developed, to which the regular rhythm might be attributed. Finally, a short survey is given of the literature dealing with the combination of auricular fibrillation and block, classification and treatment of this state.

References.

- de Boer, S.: Dtsch. med. Wschr. 52, 1945 (1926). — Frédéricq, L.: Arch. int. d. Physiol. 2, 281 (1904/05). — Gager, L. T.: Ann. int. Med. 5, 463 (1932), cit. Thorborg. — Kahn, R. H., Münzer, E.: Zbl. f. Herzkrkh. 4, 361 (1912), cit. Wenckebach a. Winterberg. — Kerr, J., Bender, L.: Heart 9, 269 (1921/22), cit. de Boer. — Lewis, T., Mack, E. G. Quart. J. Med. 3, 273 (1909/10) cit. Wenckebach a. Winterberg. — Müller, H.: Münch. med. Wschr. 84, 1490 (1937). — Rasmussen, H.: Klinisk elektrokardiografi, Tanum, Oslo, 1946. — Scherf, D., Boyd, L. J.: Clinical Electrocardiography, 2nd ed., Heinemann, London 1945. — Thorborg, N.: Acta med. scand. 114, 507 (1943). — Wenckebach, K. F. and Winterberg, H.: Die unregelmässige Herzthätigkeit, Engelmann, Leipzig, 1927. — Yater, W. M.: Arch. int. Med. 62, 1 (1938).

Centre pour l'étude et le traitement des tumeurs, Palerme (Italie).
(Dirigé par: Mr. le Prof. Maurice Ascoli.)

Sur la différentiation des sérums ictériques néoplasiques et non néoplasiques.

Par

Dr. VINCENT MUTOLO.¹

(Ce travail est parvenu à la rédaction le 21 Janvier 1948.)

Dans le cours de recherches systématiques poursuivies par notre école sur les sérums néoplasiques (1), on a étudié un simple procédé qui nous rend possible de reconnaître aisément, parmi les sérums ictériques, ceux des sujets porteurs de néoplasmes malins.

En voici la technique:

On mélange, dans une éprouvette de 75 mm de longueur et 10 de diamètre, 1 cc de sérum avec 2 d'éther sulfurique; après l'avoir bouchée avec bouchon de liège, on lui imprime une centaine de mouvements de haut en bas. Si le malade dont on examine le sérum est porteur d'un néoplasme malin, l'éther, qui se sépare spontanément après un court délai, est de couleur jaune. Très souvent on peut reconnaître de suite cette couleur: cependant il est préférable répéter la lecture après deux heures.

L'application pratique de la méthode a donné, dans grand nombre de cas, des indications diagnostiques décisives.

D'Alessandro et Indovina (2), ont observé et décrit un cas dans lequel l'épreuve de l'éther a été faussement positive, tout en étant la malade exempte de tout néoplasme: il s'agissait d'une femme enceinte de 9 mois, ictérique. Cependant, après l'accouchement, perdurant l'état ictérique, la recherche, répétée, donna, cette seconde fois, un résultat négatif. On doit penser évidemment qu'on se trouvait en présence d'un cas complexe de positivité passagère, due à l'interférence de la grossesse sur l'ictère, et qu'il faut considérer négatif.

¹ Via Villa Florio 87, Palerme (Italie).

La valeur pratique de la réaction a été pleinement confirmée par Albers et Merten (3). Ces AA. eurent des résultats constamment négatifs par 18 sérums de malades ictériques non cancéreux et positifs par 20 sérums néoplasiques sur 22 examinés; les 2 négatifs étaient respectivement un cancer de la tête du pancréas et un cancer de l'utérus avec métastases hépatiques.

Dans les dernières années j'ai pratiqué dans notre Institut l'épreuve de l'éther sur 121 sérums ictériques, dont 22 néoplasiques. Les résultats sont rapportés dans les tableaux suivants.

Tableau 1.

Diagnostic	nombre des observations	épreuve de l'éther
hépatite syphilitique	16	négatif
hépatite infectieuse	1	négatif
ictère catarrhal	37	négatif
cholécystite	2	négatif
angiocholite	13	négatif
cirrhose hépatique	15	négatif
lithiase biliaire	11	négatif
ictère hémolytique	4	négatif

Tableau 2.

n° prot.	diagnostic	épreuve de l'éther	observations
3	cancer du foie	positif	exploration chirurgicale
4	cancer de la vésicule biliaire	positif	opération
14	cancer de l'ampoule de Vater		opération
16	cancer du foie	positif	exploration chirurgicale
32	cancer de l'estomac	positif	métastase hépatique
38	cancer de la tête du pancréas	positif	exploration chirurgicale
51	cancer du foie	positif	exploration chirurgicale
52	cancer du sein	positif	métastase hépatique
53	cancer de l'ampoule de Vater	positif	opération
56	cancer du sein	positif	métastase hépatique
60	cancer du foie	positif	diagnostic clinique
63	cancer de l'ampoule de Vater	positif	opération
68	cancer de l'estomac	positif	métastase hépatique
74	cancer du foie	positif	exploration chirurgicale
75	cancer du foie	positif	exploration chirurgicale
80	cancer de l'ampoule de Vater	positif	opération
81	cancer du foie	positif	diagnostic clinique
82	cancer du foie	positif	diagnostic clinique
93	cancer de l'ampoule de Vater	positif	opération
101	cancer de l'estomac	positif	métastase hépatique
107	cancer du foie	positif	diagnostic clinique
112	cancer de l'ampoule de Vater	positif	opération

Tableau 3.

	D'Alessandro Indovina		Albers Merten		Nos Cas		Totals		Pourcentage	
	positif	négatif	positif	négatif	positif	négatif	positif	négatif	positif	négatif
néoplasiques.....	—	—	20	2	22	—	42	2	95.46 %	4.54 %
non néoplasiques .	—	1	—	18	—	99	—	118	0 %	100 %

De l'examen de la 3^{me} table, où nous prenons en considération tous les cas dans lesquels a été pratiqué l'épreuve de l'éther et desquels on connaît les résultats, on peut conclure que la réaction a donné constamment des réponses négatives pour les sérums provenant de malades ictériques mais non cancéreux, et positives dans le 95.46 % des cancéreux.

On peut donc affirmer que, si l'on excepte des cas assez rares, cette épreuve est un moyen apte à établir avec certitude si un sérum ictérique appartient à un malade porteur d'une néoplasie maligne.

Summary.

The ether test for diagnosis of cancer in jaundiced patients (traction of bilirubin from the sera) is studied.

Sera from patients with malignant neoplasm give 100 % of positive results.

Other human sera, tested by the same technique, are uniformly negative.

Références.

1. Ascoli M.: Klin. Wchnschr. 14, 1593, '35. — 2. D'Alessandro, G. & Indovina, R.: Biochim. e terap. sper. 22, 298, '35. — 3. Albers, D. & Merten, R.: Ztschr. f. Krebsforsch. 49, 375, '39.

Acta Medica Scandinavica.

Index of Supplementary Volumes published 1921—1948.

- I. *Aksel O. Haneborg*: The effects of alcohol upon digestion in the stomach. — 1921.
- II. *Olle P:son Reuterwall*: Über die Elasticität der Gefäßwände und die Methoden ihrer näheren Prüfung. — 1921.
- III. Verhandlungen des X. Nordischen Kongresses für innere Medizin zu Helsingfors 30. Juni—2. Juli 1921. — 1922.
- IV. *Karen Marie Hansen*: Investigations on the blood sugar in man. Conditions of oscillations, rise and distribution. — 1923.
- V. *Leonard Brahme*: Arsen in Blut und Cerebrospinalflüssigkeit. — 1923.
- VI. *Harald A. Salvesen*: Studies on the physiology of the parathyroids. — 1923.
- VII. Rapports et comptes rendus du onzième congrès de médecine des Pays du Nord tenu à Kristiania du 3 au 5 juillet 1923. — 1924.
- VIII. *Rolf Hattchöl*: Blood sugar studies, with special regard to the threshold of glucosuria in diabetes mellitus and benign chronic glycosuria. — 1924.
- IX. *Sizlen Hesser*: Serological studies of human red corpuscles. — 1924.
- X. *Johannes Helweg*: Sciatica or myopathia e labore of the posterior region of the leg. — 1925.
- XI. *Ernst B. Salén*: Studien über die Kältenhämoglobinurie. — 1925.
- XII. *Gösta Ekehorn*: Syphilis fetuum, a critical study of the syphilitic endometritis of the secundines, and of the presence, nature functions and development of the antibody-producing tissues of the fetal organism. — 1925.
- XIII. *Hans Davide*: Action of antifibrinogen serum on red corpuscles. — 1925.
- XIV. *Johannes Wahlberg*: Das Thyreotoxikosesyndrom und seine Reaktion bei kleinen Joddosen. — 1926.
- XV. *Adolf F. Lindblom*: Über die Funktionsfähigkeit der mit Pneumothorax artificialis behandelten Lunge nach ihrer Wiederentfaltung. — 1926.
- XVI. Rapports et comptes rendus du douzième congrès de médecine des Pays du Nord tenu à Stockholm du 27 au 29 août 1925. — 1926.
- XVII. *Fredrik Leegaard*: Researches regarding the haemodynamics in rabbits in normal condition and during experimental pneumonia. — 1926.
- XVIII. *Marlin Odin*: Studien über die Säureproduktion bei Diabetes mellitus. — 1927.
- XIX. *Eskil Kylin*: Der Gehalt des Blutes an Calcium und Kalium. — 1927.
- XX. *Nanna Svartz*: Etude sur les bactéries intestinales iodophiles et spécialement sur les clostridies iodophiles. — 1927.
- XXI. *Eggert Möller*: Clinical investigations into the basal metabolism in diseases of the thyroid gland. — 1927.

- XXII. *Gustaf A. Lindström*: An experimental study of myelotoxic sera. Therapeutic attempts in myeloid leukaemia. — 1927.
- XXIII. *Ulrik Quensel*: Zytologische Untersuchungen von Ergüssen der Brust- und Bauchhöhlen mit besonderer Berücksichtigung der karzinomatösen Exsudate II. — 1928.
- XXIV. *Otto Jervell*: Investigation of the concentration of lactic acid in blood and urine. — 1928.
- XXV. *Albert Grönberg*: Beitrag zur Kenntnis der klinischen Verwertbarkeit des Holmgrenschen Frontalreflexes. — 1928.
- XXVI. *Rapports et comptes rendus du treizième congrès de médecine des Pays du Nord tenu à Copenhague du 30 Juin au 1 Juillet 1927.* — 1928.
- XXVII. *Haquin Malmros*: A study of glucosuria with special reference to the interpretation of the incidental finding of a positive reduction test. — 1928.
- XXVIII. *Claes Grill*: Kavernenstudien. Physikalisch-diagnostische Gesichtspunkte betreffend die Symptomatologie der kavernösen Lungentuberkulose. — 1929.
- XXIX. *Olaf Bang*: Klinische Urobilinstudien. — 1929.
- XXX. *Folke Lindstedt*: Über die Natur der muskelerheumatischen (Myalgischen) Schmerzsymptome. — 1929.
- XXXI. *Gustav Nylin*: Periodical variations in growth, standard metabolism and oxygen capacity of the blood in children. — 1929.
- XXXII. *Johs. Mygge*: Etude sur l'écllosion épidémique de l'influenza. — 1930.
- XXXIII. *Anders Kristenson*: Zur Kenntnis der lokalisierten Thrombenbildungen in der Vena iliaca communis sinistra. — 1930.
- XXXIV. *Verhandlungen des 14. Nord. Kongresses f. innere Medizin zu Helsingfors 28.—30. Juni 1929.* — 1930.
- XXXV. *Alexander Jarotzky*: Zur diätetischen Behandlung des runden Geschwürs des Magens und des Duodenums. — 1930.
- XXXVI. *Gösta Ekehorn*: On the principles of renal function. — 1931.
- XXXVII. *Oline Christensen*: Pathophysiology of hunger pains. — 1931.
- XXXVIII. *Erik Lundberg u. Stina Thysehus-Lundberg*: Beitrag zur Kenntnis des innersekretorischen Gleichgewichtsmechanismus. — 1931.
- XXXIX. *Olaf Romcke*: Der Blutzucker im älteren Alter, insbesondere bei hypertensiven Zuständen. — 1931.
- XL. *Birger Strandell*: Pernicious anemia. A study of 117 cases. — 1931.
- XLI. *Helge Lublin*: On the late symptoms after gastroenterostomy and resection of the stomach (Billroth II) for gastric and duodenal ulcer. — 1931.
- XLII. *Ejnar Jarlov*: The clinical types of abnormal obesity. — 1932.
- XLIII. *Hans Kjærgaard*: Spontaneous pneumothorax in the apparently healthy. — 1932.
- XLIV. *E. Melkersson*: Etudes cliniques sur la réaction myodystonique. — 1932.
- XLV. *Birger Enocksson*: A study of the reducing power of the blood with special reference to some gastro-intestinal diseases and their diagnosis. — 1932.
- XLVI. *Snorre Wohlfahrt*: Die vordere Zentralwindung bei Pyramidenbahnläsionen verschiedener Art. — 1932.
- XLVII. *Helge Nyman*: Studien über Fälle, die mit Achylie resp. Hypochylie assoziiert sind. — 1932.
- XLVIII. *Stig Lindgren*: Eine Studie über depressive Sekretionsanomalien des Magens. — 1932.
- XLIX. *A. Lichtenstein*: Agranulozytose. — 1932.
- L. *Proceedings of the fifteenth Scandinavian congress for internal medicine held in Oslo from 29th June to 1st July 1931.* — 1932.
- LI. *Bertel von Bonsdorff*: Zur Methodik der Blutdruckmessung. — 1932.
- LII. *Gustav Nylin*: Clinical tests of the function of the heart. — 1933.
- LIII. *Gustaf F. Göthlin*: Determination of the antiscorbutic potency of vegetable products. — 1933.

- LIV. *William Thune Andersen*: Studies on blood sugar and glycosuria in exophthalmic goitre. — 1933.
- LV. *Birger Strandell*: On the influence of exercise on the blood sugar especially in connection with glucose ingestion. — 1934.
- LVI. *Stig Björkman*: Bronchospirimetrie. — 1934.
- LVII. *Arvo Vesa*: Studien über Diabetes mellitus unter Anwendung von zweistündlichen bei Tag und Nacht entnommenen Blutzucker- und Harnproben. — 1934.
- LVIII. *Mons-Christian Ehrström*: Eine Studie über die Bedeutung von Totalserumkalziumanalysen in der Klinik. — 1934.
- LIX. Proceedings of the sixteenth Scandinavian congress for internal medicine held in Upsala from the 6th to 8th June 1933. — 1934.
- LX. *G. Nylander*: Beiträge zur Kenntnis der Anämie bei den diffusen Nierenkrankungen. — 1935.
- LXI. *Gunnar Edström*: Studies in natural and artificial atmospheric electric ions. — 1935.
- LXII. *Torsten G:son Hafström*: Takatas modifizierte Sublimatsuchreaktion am Blutserum als Diagnostikum bei Leberkrankheiten. — 1935.
- LXIII. *Snorre Wohlfahrt und Gunnar Wohlfart*: Mikroskopische Untersuchungen an progressiven Muskelatrophien. — 1935.
- LXIV. *Elsa Segerdahl*: Über Sternalpunktionen. — 1935.
- LXV. *I. L. Blum*: Working test as clinical method for determining the function of the lungs. — 1935.
- LXVI. *Tor Engeström*: Beitrag zur Kenntnis der Magensaftacidität und der Verdünnungssekretion des Magens. — 1935.
- LXVII. *Ragnar Gärdstam*: Über Harnsäureausscheidung bei Kreatinbelastung. — 1935.
- LXVIII. *Anton Jerrell*: Elektrokardiographische Befunde bei Herzinfarkt. — 1935.
- LXIX. *Gustav Nylin*: The physiology of the circulation during puberty. — 1935.
- LXX. *Ruth Svensson*: Studies on human intestinal protozoa. — 1935.
- LXXI. *Birger Strandell*: Experiments to isolate the antianemic principle of the liver. — 1935.
- LXXII. *Karl Lunding*: The symptomatology of diverticulum formations of the colon. — 1935.
- LXXIII. *Robert Hansson*: Report on therapeutic tests in certain forms of tuberculosis with an antituberculosis serum prepared by J. Reenstierna. — 1936.
- LXXIV. *Hjalmar Holmgren*: Studien über 24-stunden-rhythmische Variationen des Darm-, Lungen- und Leberfetts. — 1936.
- LXXV. *Karl Schaffer und Desiderius Miskolczy*: Anatomische Wesensbestimmung der hereditärorganischen Nerven-Geisteskrankheiten. — 1936.
- LXXVI. *Jens Bing*: Studies on proteinuria «albuminuria». — 1936.
- LXXVII. *Esben Kirk*: Amino acid and ammonia metabolism in liver diseases. — 1936.
- LXXVIII. Rapports et comptes rendus du dix-septième congrès de Médecine des Pays du Nord tenu à Copenhague du 27 au 29 Juin 1935. — 1936.
- LXXIX. *Hans Silveer*: Studien über die N-Ausscheidung im Harn bei Einschränkung des Kohlehydratgehaltes der Nahrung ohne wesentliche Veränderung des Energiengehaltes derselben. — 1937.
- LXXX. *Chr. M. F. Sinding-Larsen*: On the collapse treatment of pulmonary tuberculosis. — 1937.
- LXXXI. *Hugo Jelke*: Ein mit A. T. 10 behandelter Fall von idiopathischer Tetanie samt einer Übersicht über die Tetanien mit besonderer Hinsicht auf Pathogenese und Therapie. — 1937.
- LXXXII. *Jan Waldenström*: Studien über Porphyrie. — 1937.

- LXXXIII. *Hugo Engleson*: Dysenteriestudien. Eine historisch-epidemiologische Untersuchung über die Dysenterie in Kronobergs Län und Blekinge, sowie in Teilen von Kristianstads und Hallands Län in Schweden in den Jahren 1749—1830 mit besonderer Berücksichtigung der Sterblichkeit und Verbreitungsweise. — 1937.
- LXXXIV. *Knud Brochner-Mortensen*: Uric acid in blood and urine. — 1937.
- LXXXV. *John Reenstierna*: A fourth orientation on the therapeutic value of an anti-leprosy serum. — 1937.
- LXXXVI. *Hans Jacob Ustvedt*: Ueber die Untersuchung der musikalischen Funktionen bei Patienten mit Gehirnleiden, besonders bei Patienten mit Aphasie. — 1937.
- LXXXVII. *A. L. Tchijevsky*: L'aéroionisation comme facteur physiologique et thérapeutique, et comme un nouvel élément sanitaire-hygiénique de l'air conditionné. — 1938.
- LXXXVIII. *Eero Ponteva*: Über die Resultate der Diabetesbehandlung in Finnland. — 1938.
- LXXXIX. Verhandlungen des achtzehnten nordischen Kongresses für innere Medizin zu Helsingfors 29. Juni—1. Juli 1937 herausgegeben von Dozent, Dr. Med. E. Adlercreutz. — 1938.
- XC. Medical and physiological papers dedicated to Dr. H. C. Hagedorn. — 1938.
- XCI. *Viggo Thomsen*: Studies of trauma and carbohydrate metabolism with special reference to the existence of traumatic diabetes. — 1938.
- XCII. *Roald Opsahl*: Zur Pathogenese der arteriellen Hypertension. — 1938.
- XCIII. *Gustav Nylin*: The practical applicability of the cardio-pulmonary function test. — 1938.
- XCIV. *Johannes Wahlberg*: Studien über die Schilddrüsenkrankheiten in Finnland. — 1938.
- XCV. *Bengt Ihre*: Human gastric secretion. A quantitative study of gastric secretion in normal and pathological conditions. — 1938.
- XCVI. *Erik Gripwall*: Zur Klinik und Pathologie des hereditären hämolytischen Ikterus. — 1938.
- XCVII. *Torsten Lindqvist*: Studien über Vitamin A beim Menschen. — 1938.
- XCVIII. *Birger Jönsson*: Zur Epidemiologie der Kinderlähmung. — 1939.
- XCIX. *Georg C. Brun*: Cholesterol content of the red blood cells in man. — 1939.
- C. *Eric Jonsson*: Studien über experimentelle Arthritiden und Kardiiden. Ein Beitrag zur Frage der pathogenetischen Bedeutung endokriner Faktoren bei dem sogenannten Gelenkrheumatismus. — 1939.
- CI. *Jarl Forssell*: Morphologische Veränderungen im Knochenmark und Blut bei akuten Blutungsanämien. — 1939.
- CII. *I. Lundholm*: Hereditary hypochromic anemia. — 1939.
- CIII. *Karl Evang* and *Otto Galtung Hansen*: An inquiry into the diet of 301 poorly situated families in Norway. — 1939.
- CIV. *Guido Tötterman*: Über Sternalmark und Blut bei Wurmträgern. — 1939.
- CV. *Erling Wang*: Clinical and experimental investigations on the creatine metabolism. — 1939.
- CVI. *Knut Liedholm*: Studien über das Verhalten des Venendruckes beim valsalvaschen Versuch. — 1939.
- CVII. *Jean Lequime*: Le débit cardiaque. — 1940.
- CVIII. Verhandlungen der zweiten Konferenz der internationalen Gesellschaft für biologische Rhythmusforschung am 25. und 26. August 1939, Utrecht (Holland). — 1940.
- CIX. *Per Hedenius*: Über wahre Metachromasie der weissen Blutkörperchen. — 1940.

- CX. *Hans Difs*: Beiträge zur Diagnostik der Vitamin-C-Mangelkrankheit. — 1940.
- CXI. *Turo Niemi*: Die Senkungsreaktion der roten Blutkörperchen bei Embolien, Thrombosen und Gehirnblutungen sowie einigen anderen Gefässkrankungen. — 1940.
- CXII. *Hall Scharium-Hansen*: Das Sternalmark bei leukämischen Krankheiten und die Genese der Monozyten. — 1940.
- CXIII. *Acta medica scandinavica*, author and subject index to vol. 52—100, and supplements 1—100, 1919—1939. — 1940.
- CXIV. *Hugo Jelke*: Über Hyperparathyreoidismus. — 1940.
- CXV. *Håkon Rasmusen*: Influence of the thyroid hormone on heart and circulation. — 1941.
- CXVI. *Jørgen H. Vogt*: The influence of some diet factors on the irritability of the skin and on the mineral contents of the skin and blood plasma in rabbits. — 1941.
- CXVII. *Fredrik Sundelin*: Die Goldbehandlung der chronischen Arthritis unter besonderer Berücksichtigung der Komplikationen. — 1941.
- CXVIII. *John Reenstierna*: Further therapeutic tests with an antileprosy serum. — 1941.
- CXIX. *Olof Nordenfjelt*: Über funktionelle Veränderungen der P. und T-Zacken im Elektrokardiogramm. — 1941.
- CXX. *Leo Noro*: Untersuchungen über die Trotyl-, Tetryl- und Knallquecksilbervergiftungen bei den Arbeitern der Munitionsfabriken Finnlands. — 1941.
- CXXI. *Aage Lachmann*: Hypoparathyroidism in Denmark. A clinical study. — 1941.
- CXXII. *Carl August Hernberg*: Die Grösse und Form der roten Blutkörperchen bei Menschen verschiedenen Alters unter physiologischen Verhältnissen. — 1941.
- CXXIII. Rapports et comptes rendus du dix-neuvième congrès de médecine des pays du Nord tenu à Oslo du 27 au 29 Juin 1939. — 1941.
- CXXIV. *Gösta Widström*: The problem of vaccination against tuberculosis. An experimental study. — 1941.
- CXXV. *Mikael Skjelderup Kobro*: Asthmatic bronchitis. A clinical, pathogenetic and therapeutic study. — 1942.
- CXXVI. *Ole K. Evensen*: Alimentary hypoglycemia after stomach operations and influence of gastric emptying on glucose tolerance curve. — 1942.
- CXXVII. *Karl Östner*: Studien über die Heparinblutsenkungsreaktion und Heparin-Citrat-Blutsenkungsreaktion. — 1942.
- CXXVIII. *Henrik O. Lagerlöf*: Pancreatic function and pancreatic disease studied by means of secretin. — 1942.
- CXXIX. *Torsten Bruce*: Die Silikose als Berufskrankheit in Schweden. Eine klinische und gewerbemedizinische Studie. — 1942.
- CXXX. *Sixten Kallner*: The cyanosis developing during treatment with sulfanilamide preparations. — 1942.
- CXXXI. *Arne Barfred*: Investigations into the biological effects of liver extracts with special reference to the gastric-stimulating principle. — 1942.
- CXXXII. *Carl-Olof Oldfjelt*: Oxygen consumption and growth and the effect of immune and normal sera. In vitro studies on two bacterial strains. — 1942.
- CXXXIII. *Per Wising*: A study of infectious mononucleosis (Pfeiffer's disease) from the etiological point of view. — 1942.
- CXXXIV. *Jørgen E. Thygesen*: The mechanism of blood sedimentation. — 1942.
- CXXXV. *Erik Hedvall*: Bovine tuberculosis in man. A clinical study of bovine tuberculosis, especially pulmonary tuberculosis, in the southernmost part of Sweden, and *Hilding Magnusson*: The relation between bovine and human tuberculosis from the veterinary point of view. — 1942.

- CXXXVI. *Paavo Maijala*: Klinische Untersuchungen über die Häufigkeit und Art der seropositiven Spätluës in Finnland. — 1942.
- CXXXVII. *Thor Sällström*: Das Vorkommen und die Verbreitung der multiplen Sklerose in Schweden. — 1942.
- CXXXVIII. *Fritz Karlström*: The Cl-ion content of the cerebrospinal fluid and its relation to the Cl-ion content of the blood. — 1942.
- CXXXIX. *Bertil Dahlberg*: The masticatory effect. A new test and an analysis of mastication in more or less defective set of teeth. — 1943.
- CXL. *Rolf Hallgren*: Epidemic hepatitis in the county of Västerbotten in northern Sweden. An epidemiological, clinical and etiological study. — 1943.
- CXLI. *Gunnar Löfström*: Nonspecific capsular swelling in pneumococci. A serologic and clinical study. — 1943.
- CXLII. *A. Rune Frisk*: Sulfanilamide derivatives. Chemotherapeutic evaluation of N¹-substituted sulfanilamides. — 1943.
- CXLIII. *Sven Gard*: Purification of poliomyelitis viruses. Experiments on murine and human strains. — 1943.
- CXLIV. *Einar Hollström*: An investigation into a yeast-like fungus isolated from patients suffering from, or suspected of, pulmonary tuberculosis. — 1943.
- CXLV. *S. Perséus*: The influence of heart glucosides, theophylline and analeptics on the cardiac output in congestive heart failure. With remarks on the acetylene methods for the determination of the arteriovenous oxygen difference. — 1943.
- CXLVI. *Mikko Virkkunen*: Untersuchungen über den Einfluss der Tonsillitis und der Tonsillektomie auf das Sternalpunktat und das Blutbild. — 1943.
- CXLVII. *Jakob Möllerström*: Das Diabetesproblem. Die rhythmischen Stoffwechselvorgänge. — 1943.
- CXLVIII. *Gunnar Dahlberg*: Mathematische Erblichkeitsanalyse von Populationen. — 1943.
- CXLIX. *Rolf Luft*: A study on hirsutism, Cushing's syndrome and precocious puberty. — 1944.
- CL. *Erik Sköld*: On hemophilia in Sweden and its treatment by blood transfusion. — 1944.
- CLI. *Uno Carlborg*: Studies of circulatory disturbances, pulse wave velocity and pressure pulses in larger arteries in cases of pseudo-xanthoma elasticum and angiod streaks. A contribution to the knowledge of the function of the elastic tissue and the smooth muscles in larger arteries. — 1944.
- CLII. *Richard F. Öhnell*: Pre-excitation. A cardiac abnormality. Pathophysiological, patho-anatomical and clinical studies of an excitatory spread phenomenon bearing upon the problem of the WPW (Wolf, Parkinson and White) electrocardiogram and paroxysmal tachycardia. — 1944.
- CLIII. *C. E. Nylund*: Über die Untersuchungstechnik bei der Bestimmung von Nachtblindheit als Symptom von Vitamin-A-Mangel und Untersuchungen über das Vorkommen von Nachtblindheit und über ihre Abhängigkeit von der Vitamin-A-Zufuhr. — 1944.
- CLIV. *Gösta Birath*: Lung volume and ventilation efficiency. Changes in collapse-treated and non-collapse-treated pulmonary tuberculosis and in pulmonectomy and lobectomy. — 1945.
- CLV. *E. V. Helander*: Über die Magensekretion bei Bothriocephalus-trägern. — 1945.
- CLVI. *Alvar Ehinger*: On the haemolytic streptococci in scarlet fever. — 1945.
- CLVII. *Nils Skiöld*: Erythema nodosum. — 1945.
- CLVIII. *Karl-Axel Ekblom*: Restless legs. — 1945.
- CLIX. *Hans Forssman*: On hereditary diabetes insipidus with special regard to a sex-linked form. — 1945.
- CLX. *Arne Lithander*: Acute adrenal insufficiency in rabbits produced by some bacterial toxins. — 1945.

- CLXI. *Ulf Borell*: On the transport route of the thyrotropic hormone, the occurrence of the latter in different parts of the brain, its effect on the thyroidea. — 1945.
- CLXII. *Börje Olhagen*: Studies on thermostabile anticomplementary human sera. — 1945.
- CLXIII. *Olle Löfgren*: Studien über den intermediären Stoffwechsel bei chronischer Polyarthrit. — 1945.
- CLXIV. *Juhani Vilkki*: Über die Henry-Seroreaktion und ihre klinische Anwendung. — 1945.
- CLXV. *Henning Vogelius*: Basal metabolism of girls and the use of metabolic standards. — 1946.
- CLXVI. *Ole Jacob Broch*: Studies on the regulation of the serum electrolytes with a survey of the water and salt metabolism in the organism. — 1946.
- CLXVII. *U. P. Kokko*: Über Flexner-Bazillen und Flexner-Dysenterie. — 1946.
- CLXVIII. *Helge Laake*: Experimental investigations of the excretory and reabsorptive functions of the renal tubules in normal and nephrotic rabbits. — 1946.
- CLXIX. *Erling Wang*: Creatine metabolism and endocrine regulation. — 1946.
- CLXX. *Liber gratulatorius Gustavo Bergmark, idibus martiis 1946 munus academicum deponenti a collegis amicis discipulis dedicatus.* — 1946.
- CLXXI. *Harry Ziliacus*: On the specific treatment of thrombosis and pulmonary embolism with anticoagulants, with particular reference to the post-thrombotic sequelae. — 1946.
- CLXXII. *Poul Bechgaard*: Arterial hypertension. A follow-up study of one thousand hypertonics. — 1946.
- CLXXIII. *Johan Rudebeck*: Clinical and prognostic aspects of acute glomerulonephritis. — 1946.
- CLXXIV. *Sven Löfgren*: Erythema nodosum. Studies on etiology and pathogenesis in 185 adult cases. — 1946.
- CLXXV. *Stina Björk*: Haemodynamic factors and retinal changes in hypertensive diseases. — 1946.
- CLXXVI. *Henrik F. Lange*: The normal plasma protein values and their relative variations. — 1946.
- CLXXVII. *Toivo Stenstam*: Peroral and intravenous galactose tests. — 1946.
- CLXXVIII. *Per Hanssen*: Diabetes Mellitus in Bergen 1925—1941. — 1946.
- CLXXIX. *Helge Petersen*: On the distribution of the morbidity of epidemic diseases with regard to age. — 1946.
- CLXXX. *Vilhelm Hallberg*: A new method for staining tubercle bacilli, applicable also to the micro-organism of leprosy and other acid-fast germs. — 1946.
- CLXXXI. *Erik Hedvall*: Tuberculosis incipiens. — 1946.
- CLXXXII. *Oluf Røe*: Methanol poisoning, its clinical course, pathogenesis and treatment. — 1946.
- CLXXXIII. *Esbén Kirk*: Acidosis. Clinical aspects and treatment with isotomic sodium bicarbonate. — 1947.
- CLXXXIV. *Esbén Kirk and Sven Ancher Kvorning*: Hypometabolism. — 1947.
- CLXXXV. *Gösta Ekehorn*: The quantitative nature of renal research and other concluding remarks. — 1947.
- CLXXXVI. *Edvard Ljungberg*: On the reabsorption of chlorides in the kidney of rabbit. — 1947.
- CLXXXVII. *Gösta Ekehorn*: Sherrington's »Endeavour of Jean Fernel» and »Man on his nature». — 1947.
- CLXXXVIII. *Gunnar Dahlberg, Erik Jorpes, Sixten Kallner and A. Lichtenstein*: Diabetes mellitus in Sweden. — 1947.
- CLXXXIX. *John Hilding Tomenius*: A study on the gastric sediment. — 1947.
- CXC. *Wilhelm T. L. Ohlsson*: A study on oxygen toxicity at atmospheric pressure with special references to the pathogenesis of pulmonary damage and clinical oxygen therapy: — 1947.

- CXCI. *Sven Erik Björkman*: The splenic circulation with special reference to the function of the spleen sinus wall. — 1947.
- CXCII. *Sven Hammarström*: Arterial hypertension. — 1947.
- CXCIII. *Lars Werkö*: The influence of positive pressure breathing on the circulation in man. — 1947.
- CXCIV. *Paul A. Owren*: The coagulation of blood. Investigations on a new clotting factor. — 1947.
- CXCV. *Gunnar Malmström*: The cardiological anoxemia test with special reference to its standardization. — 1947.
- CXCVI. *Hilding Berglund*. — 1947.
- CXCVII. *I. Blum*: Tuberculosis and pregnancy. — 1947.
- CXCVIII. *Stig Tejning*: Dietary factors and quantitative morphology of the islets of Langerhans. — 1947.
- CXCIX. *Gösta Ekehorn*: Sherrington's »Endeavour of Jean Fernel» and »Man on his nature». — 1947.
- CC. *Bo Thorell*: Studies on the formation of cellular substances during blood cell production. — 1947.
- CCI. *Herman Hortling*: The influence of electric shock and adrenalin injections on the leukopoiesis and the erythropoiesis. — 1948.
- CCII. *Ake Edlén*: Pathophysiology of peptic ulcer. — 1948.
- CCIII. *Gösta Ekehorn*: Sherrington's »Endeavour of Jean Fernel» and »Man on his nature». — 1948.
- CCIV. *Astrid Fagraeus*: Antibody production in relation to the development of plasma cells. — 1948.
- CCV. *Gunnar Wilman*: A contribution to the knowledge of the cellular content in exudates and transudates. — 1948.
- CCVI. *Comptes rendus du vingtième congrès de médecine interne des pays du Nord, réuni à Gothembourg du 27 au 29 Juin 1946*. — 1948.
- CCVII. *J. G. G. Borst*: The maintenance of an adequate cardiac output by the regulation of the urinary excretion of water and sodium chloride; an essential factor in the genesis of oedema. — 1948.
- CCVIII. *Jóhann Saemundsson*: Potassium concentration in human gastric juice. — 1948.
- CCIX. *Karl Erik Grewin*: Some supplementary leads in clinical electrocardiography. — 1948.
- CCX. *Fried Nilsson*: Anemia problems in rheumatoid arthritis. — 1948.
- CCXI. *Börje Ejrup*: Tonosillography after exercise. — 1948.
- CCXII. *Ilmari Paronen*: Reiter's disease. — 1948.
- CCXIII. *Einar Meulengracht*, in honorem. — 1948.
- CCXIV. *Axel Ström*: Examination into the diet of norwegian families during the war-years 1942—45. — 1948.
-

Publications Received.

Redaktionen sänder på anmodan böcker för recension.

- Arquivos da Universidade da Bahia.* Faculdade de Medicina. Tipografia Beneditina. Bahia. Brasil.
- Current Researches in Anesthesia & Analgesia.* Vol. 27. Nr. 4, July—August, 1948. Ohio, U. S. A.
- Revista Cubana de Laboratorio Clinico.* Vol. II. Num. 2. Abril—Mayo—Junio 1948. Habana, Cuba.
- La Tunisie Médicale.* Juillet—Août 1948, n:o 7. Tunis.
- Umberto Niccolini:* Terapia dell'urto quantistico. Prime esperienze. 23 p. Istituto Radiazioni Elettromagnetiche, Milano, 1948.
- Hanns Alexander:* Differentialdiagnostische Bilder zur Lungentuberkulose. 2., neu bearbeitete Auflage des I. und II. Teiles. 146 S. 127 Abb. Georg Thieme, Leipzig, 1948.
- J. Samuels:* Endogeneous endocrinotherapy and the causal cure of cancer. 8 p. Amsterdam, 1948.
- Papers from the IV. Medical Service of St. Erik's Hospital, Stockholm.* Vol. V. Edited by Hilding Berglund. Alb. Bonniers Boktryckeri, Stockholm, 1948.
- Hawaii Medical Journal and Inter-Island Nurses' Bulletin.* Vol. 7, nr. 6, July—August, 1948.
- Acta Medica Philippina.* Vol. IV no. 4, April—June, 1948. Manila. Philippines.
- F. Haurowitz:* Fortschritte der Biochemie 1938—1947. 364 S. Preis: Sfr. 40.—. S. Karger, Basel & New York, 1948.
- Rolv Hoyer Dahl:* Lungetuberkulosen. Grunnlaget, Utviklingen og Behandlingen i dag. 7 forelesninger våren 1948. 78 s. Olaf Norlis Forlag, Oslo 1948.
- Herbert Nathorst:* The prognosis of exudative pleurisy in children. 174 p. Acta tuberculosea scandinavica, Supplementum XVII. Ejnar Munksgaard, Copenhagen, 1948.
- Nevio Quattrin:* Gli itteri emolitici costituzionali atipici. 262 p. Prezzo: L. 1250. Edizioni Minerva Medica S. A., Torino 1948.
- Erik Hedvall:* Föreläsningar i tuberkulos. 163 sid. 21 fig. Stockholm 1948.